Synthesis of Protected Amino Hexitol Nucleosides as Building Blocks for Oligonucleotide Synthesis

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Supporting Information

ABSTRACT: A new synthesis protocol for the preparation of hitherto unknown 1',5'-anhydro-4'-amino-trityl/MMTr hexitol nucleosides has been developed. Key steps in the synthesis of the pyrimidine analogues (U and C) include the regioselective D-*allo*-hexitol oxirane and 2',4'-anhydronucleoside ring opening by uracil and azide, respectively. A different strategy using a regioselective epoxide ring opening of D-*gulo*-oxirane, followed by a S_N2 type of azidation reaction, has been adopted for the purine analogues (A and G). These compounds can be easily converted to 6'-phosphoramidites for the solid-phase synthesis of N4' → P6' phosphoramidates of amino hexitol nucleic acids (AHNA).

INTRODUCTION

Since the formulation of the antisense principle,¹ a wide variety of synthetic oligonucleotides and their conjugates have been investigated,² mainly aimed at the development of conformationally constrained oligomers^{3,4} that can inhibit gene expression by hindering the mRNA translation into proteins. A successful example is Vitravene, a phosphorothioate oligonucleotide that received marketing approval for the treatment of cytomegalovirus retinitis (CMV) infections in immunocompromised patients.⁵ Sugar, base, as well as the phosphodiester linker have been modified in this search for high affinity RNA binders.⁶ Major efforts in our and other laboratories have been devoted to the replacement of the five-membered furanose moiety with a six-membered hexopyranosyl sugar mimic such as altritol nucleic acid (ANA), 3'-O-Me ANA, mannitol nucleic acid (MNA),⁷ cyclohexa(e)ne ring [cyclohexanyl NA (D-CNA), cyclohexenyl NA (D-CeNA)], and 3'-fluoro hexitol NA (FHNA and Ara-FHNA).⁸ One of the first examples of an oligonucleotide with a six-membered sugar modification that hybridizes with RNA is represented by hexitol nucleic acid (HNA).⁹ Among the natural DNA and RNA phosphodiester linker mimics, a notable example is the N3' → P5' phosphoramidate oligonucleotide (in which the 3'-O-atom of the ribose moiety is replaced by a 3'-amino group) that increases the duplex stability by an average of 2 °C per base pair versus RNA.¹⁰

The insertion of HNA units in DNA, as well as the introduction of N3' → P5' phosphoramidate linkages, lead to duplex stabilization with RNA. These observations prompted us to explore the synthesis of hybrid molecules, that is, 1',5'-anhydro-3'-deoxy-4'-amino hexitol nucleosides (compounds 1–4, Figure 1), and the corresponding oligonucleotides. Here, we describe the synthesis of the amino hexitol nucleosides (AHNA) with the four natural RNA-nucleobases, that is, uracil

(1), cytosine (2), adenine (3), and guanine (4), as precursors, which can be easily converted to their 6'-phosphoramidites that can be used in the solid-phase synthesis of N4' → P6' phosphoramidates of HNA. In oligonucleotide synthesis, both the monomethoxytrityl (MMTr) and the trityl (Trt) can be used as protecting groups for the amino functionality, although trityl protection is easier to handle for stability reasons. These newly developed optimized synthetic strategies are useful to produce gram quantities of aminohexitol monomers that are required to explore the structural, functional, and biological properties of amino hexitol oligonucleotides.

Key transformations in the synthetic sequence are the introductions of the 4'-amino group and the nucleobases with the correct stereochemistry, as well as the regioselective introduction of orthogonal protecting groups.

For the synthesis of D- and L-1',5'-anhydrohexitol nucleosides, two main strategies have been described in the literature. An appropriate protected 1',5'-anhydro-3'-deoxy-D-*gluco*-hexitol sugar moiety is synthesized first from D-glucose removing the 1'- and 3'-hydroxyl groups, which is then coupled in S_N2 manner with nucleobases at 2'-position.¹¹ Alternatively, the protected 1',5':2',3'-dianhydro-L-*allo*-hexitol oxirane is prepared and 1',5'-anhydro-2'-(nucleobase)-2',3'-dideoxy-L-*altro*-hexitol is then obtained by epoxide ring opening with the nucleobases followed by deoxygenation of the 3'-hydroxyl group.¹² It is apparent that compounds 1–4 are accessible via two these major routes, as demonstrated in the retrosynthetic analysis (Figure 2). Reduction of a 4'-azido group is considered as an easy transformation to get an amine functionality at 4'-position. However, to introduce a 4'-azido

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Figure 1. Building blocks of sugar modified 1',S'-anhydro-3'-deoxy-4'-amino hexitol nucleic acids.

Figure 2. Retrosynthetic analysis of sugar modified dideoxy-D-alto-amino nucleosides.

Scheme 1. Synthesis of Compound 1^a

^aReagents and conditions: (a) Uracil, DBU, dry DMF, 90 °C, 4 h, 75%; (b) CS₂, 5 N NaOH, 3-bromo propionitrile, DMSO, 0 °C, 50 min, quant; (c) Bu₃SnH, AIBN, dry toluene, reflux, 3 h, 78% over two steps; (d) 80% AcOH, 85 °C, 4 h, 79%; (e) TBDPSCl, dry Py, 50 °C, 16 h, 87%; (f) MsCl, dry Py, 0 °C to rt, 16 h, 98%; (g) Et₃N, absolute EtOH, 90 °C, 16 h, 97%; (h) LiN₃, dry DMF, 135 °C, 48 h, 55%; (i) Et₃N·3HF, dry THF, 50 °C, 60 h, 85%; (j) BzCl, dry Py, 0 °C to rt, 16 h, 90%; (k) 10% Pd/C (Degussa type), H₂, absolute EtOH, rt, 16 h, 91%; (l) MMTrCl, DIPEA, dry Py, rt, 24 h, 89%; (m) 7 N NH₃ in MeOH, 55 °C, 48 h, 85%.

Scheme 2. Synthesis of Compound 2^a

^aReagents and conditions: (a) TBDPSCl, dry Py, 50 °C, 16 h, 96%; (b) POCl₃, 1,2,4-triazole, dry ACN, 0 to 35 °C, 14 h; (c) aq NH₃, dioxane, 35 °C, 16 h, 95% over two steps; (d) BzCl, dry Py, 0 °C to rt, 30 h, 75%; (e) 10% Pd/C (Degussa type), H₂, absolute EtOH, rt, 16 h, 84%; (f) TrtCl, DIPEA, dry Py, 55 °C, 120 h, 88%; (g) Et₃N·3HF, dry THF, 37 °C, 144 h, 92%.

functionality, a S_N2-type displacement of a 4'-axial (β) oriented leaving group is necessary.

The first route (route 1) involves the nucleophilic 2,4'-six-membered anhydronucleoside ring opening with an azide nucleophile at the 4'-position. 2,4'-Anhydronucleosides can be derived from a protected 1',5'-anhydro-2'-(nucleobase)-2',3'-dideoxy-*manno*-hexitol derivative via 3'-deoxygenation and oxirane ring opening (route 1a) or alternatively via S_N2-type displacement (route 1b) by an appropriate nucleobase. However, anhydronucleoside ring opening strategy is only applicable to pyrimidine nucleosides. For purine (adenine and guanine) analogues, alternative strategies have been adopted (route 2) from an axial (β) oriented hydroxyl group at 4'-position, followed by an S_N2-type inversion with an azido nucleophile. This *D*-iodose-sugar derivative can be obtained either from a *D*-*manno*-hexitol derivative by inversion of configuration at the 4'-position via an oxidation–reduction method¹³ or via a Mitsunobu reaction¹⁴ (route 2b). Alternatively, *D*-iodose-sugar derivative can be derived via epoxide ring opening of protected 1',5':2',3'-dianhydro-*D*-*gulo*-hexitol followed by Barton–McCombie deoxygenation (route 2a). The advantage of the latter approach is that the 4'-β-OH functionality can be introduced in a stereoselective way, avoiding tedious and laborious separations in Mitsunobu inversion or the potential formation of epimers in the oxidation–reduction method. Up to now, the synthesis of 1',5':2',3'-dianhydro-*D*-*gulo*-hexitol is not described in the literature. However, we presumed that it can be obtained from a (un)protected 1',5'-anhydro-2',3'-dideoxy-*D*-*threo*-hex-2'-enitol via regioselective epoxidation.

RESULTS AND DISCUSSION

Synthesis of 2'-(Uracil-1-yl)-1',5'-anhydro-2',3'-dideoxy-4'-amino Monomethoxytrityl Hexitol Nucleic Acid (U-AHNA) Building Block 1. The synthesis of U-AHNA 1 is shown in Scheme 1. 1',5'-Anhydro-4',6'-O-benzylidene-2'-deoxy-2'-(uracil-1-yl)-*D*-*altro* hexitol 6 is a known compound,¹⁵ and was obtained in 75% yield from commercially available oxirane¹⁶ 5, using slightly different reaction conditions (DBU as base in DMF at 90 °C). Reductive removal of the 3'-OH group of 6 was achieved using slightly modified Barton–McCombie conditions.¹⁷ Compound 6 was first converted to xanthate 7, which was, without any purification, immediately treated with tributyltin hydride/AIBN yielding the corresponding 1',5'-anhydrohexitol-2',3'-dideoxy-nucleoside 8 in 78% yield over two steps. Acidic cleavage of the benzylidene protecting group of the uracil derivative 8 using 80% AcOH afforded diol 9 in 79% yield. The

primary hydroxyl group of diol 9 was selectively protected using TBDPSCl in dry pyridine yielding compound 10 in 87% yield. The initial plan was to obtain azide 13 via inversion of the stereochemistry of the 4'-OH group of compound 10 by the synthesis of 2'-(uracil-1-yl)-1',5'-anhydro-2',3'-dideoxy-6'-O-TBDPS-*iodo*-hexose from 10, followed by mesylation and subsequent S_N2 displacement by an azide nucleophile. Unfortunately, this inversion of the stereochemistry by Mitsunobu reaction was unsuccessful. Inversion of configuration of the 4'-OH group by a Dess–Martin periodinane oxidation and NaBH₄ reduction sequence afforded a mixture of 4'-hydroxyl epimers, which were not separable by silica gel flash chromatography (~6:1 *cis:trans*).¹³ Therefore, azide 13 was obtained via a O²,4'-anhydronucleoside ring opening. This is equivalent to the O²,3'-anhydronucleoside intermediate for the stereoselective introduction of substituents at the 3'-position of (deoxy)furanose-sugars.^{18,19} The 4'-hydroxyl group of protected nucleoside 10 was converted to its corresponding 4'-mesyl derivative 11. Alkaline treatment (Et₃N in EtOH) afforded the 2,4'-anhydro-deoxy-*iodo*-hexose uracil derivative 12 in excellent yield (97%). 2,4'-Anhydro ring opening by nucleophilic attack of LiN₃ in DMF at 135 °C yielded the desired 2'-(uracil-1-yl)-1',5'-anhydro-2',3'-dideoxy-6'-O-TBDPS-*manno*-hexose 13 as a minor component (19%), whereas its desilylated derivative 14 was the major compound (36%). Earlier research revealed that TBDPS protecting groups are stable under reaction circumstances using NaN₃ in DMF at 60 °C for 48 h.²⁰ The exact reason for desilylation using LiN₃ is not known, but might be related to the higher temperature used and to the good nucleophilicity of azide anion and thus behaving like a pseudohalide. The 6'-OTBDPS protecting group of 13 was removed using triethylamine trihydrofluoride and the primary hydroxyl group was protected by a benzoyl group, furnishing azido nucleoside 15 (90%). Initial attempts to reduce the 4'-azido moiety of uracil-nucleoside 15 via hydrogenolysis with 10% Pd/C resulted in an intractable mixture of 4'-amino nucleoside 16, along with a nucleoside derivative with a dihydrouracil ring. The desired compound 16 was obtained in excellent yield (91%), when the Degussa-type 10% Pd/C catalyst was used. General reaction conditions that are usually used for trityl protection of amino groups (MMTrCl/Py),²¹ even at elevated temperature, resulted only in poor yields of nucleoside 17. However, using a stronger organic base like DIPEA significantly improved the yield (89%). Finally, removal of the 6'-O-benzoyl protecting groups of nucleoside 17 under modified Zemplen conditions (7 N NH₃ solution in methanol) afforded the desired 2'-(uracil-1-

Scheme 3. Synthesis of Oxirane 26^a

^aReagents and conditions: (a) NaH, BnBr, dry DMF, -20°C to rt, 16 h, 90%; (b) mCPBA, CH_2Cl_2 , -50°C to rt, 36 h, 59% for **26** and 75% for **23** + **24**.

Scheme 4. Synthesis of Compound 3^a

^aReagents and conditions: (a) Adenine, DBU, DMF, 105°C , 20 h; (b) Ac_2O , Py, rt, 24 h; (c) 7 N NH_3 in MeOH, rt, 24 h, 68% over three steps; (d) CS_2 , EtBr, NaOH, DMF, 0°C , 1 h; (e) TBTH, AIBN, toluene, reflux, 3 h, 60% over two steps; (f) BzCl , Py, 40°C , 24 h, 95%; (g) 1 M BCl_3 in CH_2Cl_2 , dry CH_2Cl_2 , -78°C to rt, 5 h; (h) 7 N NH_3 in MeOH, rt, 24 h, 86% in two steps; (i) (1) TMSCl, dry Py, 0°C to rt, 5 h; (2) BzCl , 0°C to rt, 16 h; (3) $\text{Et}_3\text{N}\cdot 3\text{HF}$, dry THF, rt, 16 h; (4) 2 N NH_3 in EtOH, 0°C , 45 min, 67% in four steps; (j) TBDPSCl, dry Py, 50°C , 24 h, 80%; (k) MsCl , dry Py, 0°C to rt, 20 h, 85%; (l) NaN_3 , dry DMF, 65°C , 72 h; (m) 10% Pd/C (Degussa type), H_2 , MeOH, rt, 16 h, 20% over two steps; (n) TrtCl , Et_3N , dry CH_2Cl_2 , 40°C , 16 h, 80%; (o) $\text{Et}_3\text{N}\cdot 3\text{HF}$, dry THF, 46°C , 72 h, 87%.

yl)-1',5'-anhydro-2',3'-dideoxy-4'-amino monomethoxytrityl hexitol nucleic acid (U-AHNA) **1** in excellent yield (85%).

Synthesis of 2'-(N⁴-Benzoyl-cytosin-1-yl)-1',5'-anhydro-2',3'-dideoxy-4'-amino Monomethoxytrityl Hexitol Nucleic Acid (C-AHNA) Building Block 2. Synthesis of C-AHNA **2** was accomplished as shown in Scheme 2. The primary hydroxyl group of compound **14** was protected as a TBDPS ether, yielding uridine derivative **13**. The uracil ring of intermediate **13** was converted to its corresponding cytosine derivative **18** in excellent yield (95%) following Reese's method²² using POCl_3 /1,2,4-triazole and NH_3 . Protection of the 4- NH_2 group of cytosine with a benzoyl group furnished **19** in 75% yield. The azide functionality of **19** was catalytically hydrogenated in the presence of 10% Pd/C (Degussa type) to yield the 4'-amino *N*⁴-benzoyl-cytosine analogue **20** (84%).

The primary 4'-amino group of cytosine nucleoside **20** was protected using TrtCl /Py/DIPEA at slightly elevated temperatures (55°C) and prolonged reaction times affording tritylated **21** (88%). Finally, desilylation of the 6'-OTBDPS group using the classical TBAF reaction conditions resulted in the formation of the desired compound **2**, along with debenzoylated cytosine analogue as a side product. This method required cumbersome and tedious purifications to remove the tetrabutylammonium salt. When other reagents for the desilylation were used (e.g., Py·HF), low yields were obtained. When a mild desilylating reagent, such as $\text{Et}_3\text{N}\cdot 3\text{HF}$, was applied, the desired 2'-(*N*⁴-benzoyl-cytosin-1-yl)-1',5'-anhydro-2',3'-dideoxy-4'-amino monomethoxytrityl hexitol nucleic acid **2** (U-AHNA) was isolated in excellent yield

Scheme 5. Synthesis of Compound 4^a

^aReagents and conditions: (a) 2-Amino-6-chloro purine, DBU, DMSO, 88 °C, 36 h, 60%; (b) 2 N NaOH, DABCO, dioxane, 90 °C, 16 h, 83%; (c) (1) TMSCl, dry Py, 0 °C to rt, 7 h; (2) isobutyric anhydride, 35 °C, 16 h; (3) Et₃N·3HF, dry THF, rt, 6 h; (4) 2 N NH₃ in EtOH, 0 °C, 2 h, 84% in four steps; (d) CS₂, 3-bromo propionitrile, 50% NaOH, DMSO, 0 °C, 2 h, 80%; (e) TBTH, AIBN, toluene, reflux, 3 h, 82%; (f) 1 M BCl₃ in CH₂Cl₂, dry CH₂Cl₂, -78 °C to rt, 7 h, 89%; (g) TBDPSCl, dry Py, 50 °C, 16 h, 75%; (h) MsCl, dry Py, 0 °C to rt, 24 h, 83%; (i) NaN₃, dry DMF, 65 °C, 72 h; (j) 10% Pd/C (Degussa type), H₂, MeOH, rt, 16 h, 30% over two steps; (k) TrtCl, Et₃N, dry CH₂Cl₂, 40 °C, 16 h, 98%; (l) Et₃N·3HF, dry THF, 46 °C, 60 h, 85%.

(92%), although the reaction proceeded rather sluggish (reaction time of 144 h, as monitored by TLC).

Synthesis of 2'-(N⁶-Benzoyladenine-9-yl)-1',5'-anhydro-2',3'-dideoxy-4'-amino Trityl Hexitol Nucleic Acid (A-AHNA) Building Block 3. Azide-mediated 2,4'-anhydro ring opening, as used for the pyrimidine nucleosides (Scheme 1), cannot be applied for the synthesis of the purine nucleoside analogues A-AHNA (3) and G-AHNA (4). In contrast, the key step here is the introduction of the 4'-azido group onto a 3'-deoxy-iodo-hexose nucleoside. As inversion of configuration of the 4'-hydroxyl group of 3'-deoxy-iodo-hexose nucleoside was cumbersome in the uridine series (Scheme 1), an alternative synthetic road was established, starting from a dibenzyl protected 1',5':2',3'-dianhydro-D-gulo-hexitol 26.

The synthesis of D-gulo-oxirane 26 is depicted in Scheme 3. 1',5'-Anhydro-2',3'-dideoxy-D-threo-hex-2-enitol 22 was accessible from β-D-galactose pentaacetate in four steps according to known procedures.^{23,24} Initial attempts in the epoxidation of unprotected 2',3'-dideoxy-2',3'-unsaturated pyranoside 22 with mCPBA resulted in a mixture of ~5:1; *syn* 23:*anti* (desired) 24 oxirane. This result arises presumably due to Henbest-type directivity of the allylic 4'-OH function, which promotes *syn* epoxidation.²⁵ Simultaneous protection of the 4'- and 6'-OH as benzylothers afforded the pyranoside 25. Epoxidation with mCPBA provided the desired *anti* oxirane 26 stereoselectively in 59% yield. A coupling constant between H-1'eq and H-2'ax of 3.4 Hz is indicative for an α-oxirane in compound 26.

The synthesis of A-AHNA 3 from benzyl protected D-gulo-oxirane 26 is shown in Scheme 4. Treatment of oxirane 26

with adenine under basic conditions (DBU in DMF) at 105 °C selectively introduces the adenine base at the 2'-position according to the Fürst–Plattner rule,²⁶ yielding a nonseparable mixture (column chromatography) of N⁹-substituted hexitol nucleoside analogue 27, along with its undesired N⁷ adduct 28 (~3:1 ratio). The mixture of 27 and 28 was treated with acetic anhydride. At this stage of the synthesis, the acetylated derivatives of 27 and 28 were separated by flash chromatography. The acetylated analogue of 27 was treated with 7 N NH₃ in MeOH furnishing pure 27 in a 68% yield over three steps. Unlike uracil xanthate 7, a careful handling of metastable adenine xanthate 29 was required for 3'-deoxygenation of 27 under modified Barton–McCombie conditions,¹⁷ furnishing the corresponding 3'-deoxynucleoside 30 in 60% yield in two steps. A wide variety of hydrogenation conditions were explored for removal of the benzyl groups, using different Pd-catalysts like 10% Pd/C, 20% Pd(OH)₂/C, Pd-black, or Pd(OAc)₂. This resulted either in the recovery of unreacted starting material (mild condition) or in the formation of undesired side products (not isolated, harsh condition), probably due to catalyst poisoning arising from traces of tin- or sulfur-byproducts transmitting from earlier step. Lewis acid-mediated debenzoylation (using boron trichloride) of 30 resulted only in 6'-mono debenzoylated product, probably due to the poor solubility of the mono debenzoylated intermediate in dichloromethane. To increase the solubility in organic solvents, the exocyclic amino group of adenine of compound 30 was benzoyl protected furnishing intermediate 31. Boron trichloride-mediated didebenzoylation of 31 afforded a ~1:1 mixture of diol 33, along with debenzoylated diol 32, formed

Figure 3. Plausible pathways of dihydropyran and azido nucleoside mixture formation.

due to acidic deprotection by HCl that was formed during workup (quenching BCl_3 with methanol). As separation of **32** and **33** by silica gel flash chromatography was difficult, the mixture was treated with 7 N NH_3 in MeOH to obtain the completely deacetylated analogue **32** as a single compound, which could be conveniently purified. At this stage of the synthesis, the formation of the desired N-9 regioisomer was confirmed by the observation of a clear HMBC correlation between H-2' and C-4 (see HMBC spectrum of compound **32** in the [Supporting Information](#)). Transient protection of 4'- and 6'-OH of **32** with TMSCl and reprotection of the amino group with a benzoyl group gave derivative **33** in 67% yield over four steps. Selective protection of the 6'-primary hydroxyl group of adenine nucleoside **33** was achieved using *tert*-butyldiphenylsilyl chloride in pyridine, furnished compound **34**. All methods to convert the 4'-hydroxyl group into a 4'-O-triflate were unsuccessful, providing either a pyridine-adduct or an eliminated side product. Therefore, a 4'-O-mesyl group was installed in 85% yield, along with a small amount of eliminated product (12%). Several reaction conditions and reagents, that is, $\text{TMSN}_3/\text{LiF}/\text{TMEDA}/\text{DMF}$, LiN_3/DMF , DPPA/DBU/THF,²⁷ DPPA/DIAD/ PPh_3/THF ,¹⁴ and NaN_3/DMF , at variable temperature were screened for a selective $\text{S}_{\text{N}}2$ -type nucleophilic 4'-azido displacement of mesyl analogue **35**. When DPPA was used as azide source, no desired product was formed at ambient temperature, whereas at elevated temperature only undesired side compounds were formed (not isolated). When TMSN_3 , LiN_3 , and NaN_3 were applied mostly unreacted starting material was recovered at ambient temperature, and at elevated temperature ($>100^\circ\text{C}$), a complex reaction mixture was formed, with the undesired dihydropyran (**37**) as a major product, along with the desired azido nucleoside **36** and a mixture of the desilylated counterparts of **36** and **37**, as well as a small fraction of the debenzoylated product. Using crown ethers to increase the nucleophilicity of the azide ion did not improve the reaction yields. Ultimately, NaN_3 in DMF at moderate temperature (65°C) for a prolonged reaction time (72 h) was found to be the optimal condition, reducing the formation of eliminated, desilylated, and debenzoylated side products. After workup, the crude reaction mixture was treated with TBDPSCl/Py for resilylation. Having the same R_f , the separation of azido nucleoside **36** and dihydropyran **37** was not possible via column chromatography. The mixture of **36** and **37** was subjected to hydrogenation under 10% Pd/C (Degussa type), and the resulting 4'-amino nucleoside was purified at this stage to furnishing desired **38** in 20% yield over two steps. Tritylation ($\text{TrtCl}/\text{Et}_3\text{N}/\text{CH}_2\text{Cl}_2$) yielded the 4'-amino trityl nucleoside **39**. Finally, desilylation with $\text{Et}_3\text{N}\cdot 3\text{HF}$ afforded target compound A-AHNA **3** in excellent yield (87%). The axial orientation of the adenine nucleobase of compound **3** was derived from the 3J coupling constant of 3.1 Hz between H-1'ax and H-2'eq.

Synthesis of 2'-(*N*²-Isobutyrylguanin-9-yl)-1',5'-anhydro-2',3'-dideoxy-4'-amino Trityl Hexitol Nucleic Acid (G-AHNA) Building Block 4. Similar as for the preparation of A-AHNA, the synthesis of G-AHNA **4** started from benzyl protected D-gulo-oxirane **26** as shown in [Scheme 5](#). Different methods have been described in the literature to obtain guanine nucleosides via the epoxide opening of oxirane D-allitol **5** using the lithium salt of *N*²-acetyl-2-amino-6-[2-(trimethylsilyl)ethoxy]purine¹⁵ (45%) or using the sodium salt of 2-amino-6-chloropurine/*Aliquat* 336/ K_2CO_3 ²⁸ to improve the yield (70%). Unfortunately, these methods proved to be low yielding for epoxide opening of D-gulo-oxirane **26**. Using DBU (2.3 equiv) as base and 2-amino-6-chloropurine as nucleophile (1.2 equiv) in DMF at 108°C resulted in only 41% of the desired compound **40**, accompanied by typical side products, such as the N-9-substituted regioisomer, chloro-substituted N-dimer, and *N,N*-dimethyl adducts. By switching to DMSO as solvent, reducing the DBU amount (1.3 equiv), and lowering the reaction temperature to 90°C , the formation of side products was minimalized and the desired compound **40** was obtained in 60% yield. The isolation of the desired N-9 isomer was confirmed by a clear HMBC correlation between H-2' and C-4 (see [Supporting Information](#) for compound **40**). The 6-chloro-2-aminopurine base was converted into the guanine base by NaOH treatment, followed by the transient protection of 3'-OH of **41** with TMSCl and protection of the 2-amino group as an isobutyryl group to furnish **42** in excellent yield. Barton–McCombie-mediated deoxygenation of **42**, via xanthate **43**, furnished compound **44** (82%) along with recovered starting material **42** (14%). Lewis acid-mediated cleavage of both benzyl groups, followed by selective 6'-O-silylation with TBDPSCl and 4'-O-mesylation, provided compound **47** in excellent yield.

Azidation yielded a nonseparable mixture of the desired azido nucleoside **48** as minor component, whereas the major compound was dihydropyran **49**. Hydrogenolytic azide reduction of the mixture furnished the 4'-amino guanine nucleoside **50** in 30% yield over two steps. The plausible formation of dihydropyran as a major product over azido analogue (minor) is described in [Figure 3](#). Because of the good leaving group ability of OMs group, the transition state exhibits two possibilities: either a $\text{S}_{\text{N}}2$ -type nucleophilic attack of N_3^- ion or an E_2 -type elimination. N_3^- ion is also known for good nucleophilicity, but an $\text{S}_{\text{N}}2$ -type N_3^- attack on secondary carbon (4'-) is less favored probably due to steric hindrance. Although the azide ion is a weak base, at elevated temperature it might advocate for the E_2 -type elimination in a perfectly oriented *trans* antiperiplanar geometry of TS. Tritylation of the 4'-amino group and subsequent desilylation of the TBDPS group gave access to the desired G-AHNA **4** in excellent yield. The small coupling constant 3J of 2.9 Hz between H-1'ax and H-2'eq of compound **4** confirmed the axial position of the nucleobase.

CONCLUSIONS

In this Article, an accessible and optimized protocol for the synthesis of the hitherto unknown 1',5'-anhydro-4'-amino-trityl/MMTr hexitol nucleosides **1–4** has been developed. The synthesis of the pyrimidine nucleoside U-AHNA **1** was accomplished via a regioselective epoxide and a 2',4'-anhydronucleoside ring opening by uracil and an azide anion, respectively, with an overall yield of 11% over 13 linear steps starting from D-allo-hexitol oxirane **5**. The other pyrimidine nucleoside C-AHNA **2** was obtained via conversion of the uracil base of compound **1** in the cytosine base yielding compound **2** with an overall yield of 8.3% over 15 linear steps starting from oxirane **5**. The purine nucleosides were prepared via a different strategy. Epoxide ring opening of D-gulo-oxirane **26** (accessible from β -D-galactose pentaacetate), followed by an S_N2-type azidation, afforded A-AHNA **3** with an overall yield of 0.8% over 28 linear steps and G-AHNA **4** with an overall yield of 1.4% over 21 linear steps, respectively. Compounds **1–4** are the immediate precursors of AHNA phosphoramidite building blocks. Hence, access to gram-scale quantities of 1',5'-anhydro-4'-amino-trityl/MMTr hexitol nucleosides monomers paves the way for constructing N4' → P6' phosphoramidate oligonucleotide of AHNA.

EXPERIMENTAL SECTION

General Information. For all reactions, either analytical grade or anhydrous solvents were used. All moisture-sensitive reactions were performed using oven-dried glassware (115 °C) under a nitrogen or an argon atmosphere. All heating reactions were performed in silicon oil bath, and reaction temperatures are reported as bath temperatures. Precoated aluminum sheets with silica (254 nm) were used for TLC. Compounds were visualized with UV light ($\lambda = 254$ nm). Products were purified by flash chromatography on ICN silica gel 40–60 μ , 60 Å. Melting points were recorded with a Stuart SMP20 melting point apparatus. ¹H and ¹³C spectra were recorded on either Bruker Avance 300, 500, or 600 MHz spectrometers. Compounds were characterized using 2D NMR (H-COSY, HSQC, HMBC, ROESY, and NOESY) techniques. For structural assignments, NMR signals of protons and carbons for sugar and base moieties are designated with and without a prime, respectively. Chemical shifts were referenced to residual solvent signals at δ H/C 7.26/77.16 (CDCl₃), 3.31/49.00 (CD₃OD), 4.79 (D₂O), and 2.50/39.52 (DMSO-*d*₆) relative to TMS as internal standard wherever applied. Coupling constants are stated in hertz (Hz) and were directly obtained from the spectra. NMR splitting patterns are designated as s (singlet), d (doublet), dd (doublet of doublet), t (triplet), q (quartet), m (multiplet), br (broad), and apparent (app). High-resolution mass spectra (HRMS) were obtained on a quadrupole orthogonal acceleration time-of-flight mass spectrometer (Synapt G2 HDMS, Waters, Milford, MA). Samples were infused at 3 μ L/min, and spectra were obtained in positive (or negative) ionization mode with a resolution of 15 000 (fwhm) using leucine enkephalin as lock mass.

1',5'-Anhydro-4',6'-O-benzylidene-2'-deoxy-2'-(uracil-1-yl)-D-alto-hexitol (6). To a stirred mixture of oxirane **5** (30.5 g, 130 mmol) and uracil (33.6 g, 300 mmol) in dry DMF (500 mL) was added DBU (58.2 mL, 390 mmol), and the resulting mixture was heated at 90 °C under inert atmosphere for 4 h. The reaction was monitored with TLC; upon completion, the reaction mixture was cooled and the volatiles were removed under reduced pressure. The residue was diluted with ice–water (800 mL) and neutralized with 1 N HCl at 0 °C. The aq layer was extracted with CH₂Cl₂ (3 × 600 mL). The combined organic layer was dried over Na₂SO₄, filtered, and concentrated in vacuo, and the resulting crude residue was purified by column chromatography on silica gel (*R*_f = 0.41; 10% MeOH in CH₂Cl₂; gradient CH₂Cl₂/MeOH, 99:1, v/v; 49:1, v/v; 97:3, v/v) to afford **6** (33.7 g, 75%) as a white semisolid. The NMR

spectral data are in accord with the literature.¹⁵ HRMS (ESI-TOF) *m/z*: [M + H]⁺ calcd for C₁₇H₁₈N₂O₆, 347.1237; found, 347.1238.

1',5'-Anhydro-4',6'-O-benzylidene-2',3'-dideoxy-2'-(uracil-1-yl)-D-alto-hexitol (8). To a stirred solution of compound **6** (30 g, 86.67 mmol) in DMSO (350 mL) were added aq. NaOH (5 N; 91.5 mL) followed by CS₂ (91.5 mL) at 0 °C. The resulting dark brown mixture was stirred for 20 min, and then 3-bromopropionitrile (219 mL) was added slowly to the reaction mixture. After being stirred for 45 min, the volatiles were evaporated in reduced pressure, and the crude residue was diluted with ice cold water (1.6 L). The aqueous layer was extracted with CH₂Cl₂ (3 × 600 mL), and the combined organic layer was washed with saturated NH₄Cl solution (3X), dried over Na₂SO₄, and concentrated in vacuo to give crude **7**, which was used for the next step without further purification. HRMS (ESI-TOF) *m/z*: [M + H]⁺ calcd for C₂₁H₂₁N₃O₆S₂, 476.0944; found, 476.0941.

To a stirred refluxing solution of **7** in anhydrous toluene (700 mL) under argon atmosphere were added nBu₃SnH (58.3 mL, 216.7 mmol) and a solution of AIBN (2.13 g, 13 mmol) in anhydrous toluene (100 mL) dropwise over 2.5 h. After the addition was over, the reaction mixture was refluxed for an additional 30 min. The reaction mixture was then cooled, concentrated under reduced pressure, and crude residue was purified by column chromatography on silica gel (*R*_f = 0.49; 10% MeOH in CH₂Cl₂; gradient CH₂Cl₂/MeOH, 99:1, v/v; 99:1, v/v; 19.7:0.3, v/v) to provide **8** (22.3 g, 78% over two steps) as a white solid. Mp 196 °C. ¹H NMR (600 MHz, DMSO-*d*₆) δ 11.33 (br s, 1H, NH-3), 7.93 (d, *J* = 8.1 Hz, 1H, H-6), 7.41–7.34 (m, 5H, Ar-H Ph), 5.66–5.64 (m, 2H, H-5 and CH-benzylidene), 4.56–4.54 (m, 1H, H-2'), 4.21–4.18 (m, 2H, H-6' and H-1'_{eq}), 3.99 (dd, *J* = 13.6, 3.6 Hz, 1H, H-1'_{ax}), 3.79–3.73 (m, 2H, H-4' and H-6''), 3.47–3.43 (m, 1H, H-5'), 2.20–2.16 (m, 1H, H-3'_{eq}), 2.02–1.97 (m, 1H, H-3'_{ax}); ¹³C NMR (150 MHz, DMSO-*d*₆) δ 163.3 (C-4), 150.9 (C-2), 142.7 (C-6), 137.7 (*ipso* C-Ph), 128.8 (*p*-C-Ph), 128.0 (*m*-C-Ph), 126.2 (*o*-C-Ph), 101.1 (C-5), 100.9 (CH-benzylidene), 73.3 (C-5'), 73.1 (C-4'), 68.0 (C-1'), 67.9 (C-6'), 51.0 (C-2'), 31.9 (C-3'); HRMS (ESI-TOF) *m/z*: [M + H]⁺ calcd for C₁₇H₁₈N₂O₅, 331.1288; found, 331.1288.

1',5'-Anhydro-2',3'-dideoxy-2'-(uracil-1-yl)-D-alto-hexitol (9). A stirred solution of compound **8** (22 g, 66.6 mmol) in 80% AcOH (500 mL) was heated at 85 °C for 4 h. The reaction mixture was then cooled and concentrated to dryness in vacuo, and the residue was coevaporated with a mixture of toluene and EtOH (1:1 v/v, 3 × 100 mL). The resulting crude residue was purified by column chromatography on silica gel (*R*_f = 0.16; 15% MeOH in CH₂Cl₂; gradient CH₂Cl₂/MeOH, 19:1, v/v; 9:1, v/v; 17:3, v/v) to yield **9** (12.7 g, 79%) as a white solid. ¹H NMR (500 MHz, DMSO-*d*₆) δ 11.26 (br s, 1H, NH-3), 8.01 (d, *J* = 8.1 Hz, 1H, H-6), 5.54 (dd, *J* = 8.1, 3.4 Hz, 1H, H-5), 4.91, 4.59 (2 × br s, 2 × 1H, OH-6' and OH-4'), 4.48 (br s, 1H, H-2'), 4.02 (d, *J* = 13.2 Hz, 1H, H-1'_{eq}), 3.75 (dd, *J* = 13.1, 3.5 Hz, 1H, H-1'_{ax}), 3.66 (dd, *J* = 11.9, 2.4 Hz, 1H, H-6'), 3.56–3.51 (m, 2H, H-6'' and H-4'), 3.14–3.11 (m, 1H, H-5'), 2.08 (d, *J* = 13.9 Hz, 1H, H-3'_{eq}), 1.76–1.70 (m, 1H, H-3'_{ax}); ¹³C NMR (125 MHz, DMSO-*d*₆) δ 163.2 (C-4), 150.9 (C-2), 143.2 (C-6), 100.7 (C-5), 82.6 (C-5'), 67.0 (C-1'), 60.7 (C-4'), 60.5 (C-6'), 50.5 (C-2'), 35.2 (C-3'); HRMS (ESI-TOF) *m/z*: [M + H]⁺ calcd for C₁₀H₁₄N₂O₅, 243.0975; found, 243.0974.

1',5'-Anhydro-6'-O-tert-butylidiphenylsilyl-2',3'-dideoxy-2'-(uracil-1-yl)-D-alto-hexitol (10). TBDPSCI (27.3 mL, 104.9 mmol) was added slowly to a stirred solution of compound **9** (12.7 g, 52.4 mmol) in anhydrous pyridine (250 mL), and the resulting reaction mixture was heated at 50 °C for 16 h. The reaction mixture was then cooled to 0 °C and neutralized with 5% NaHCO₃ solution. The aq layer was extracted with CH₂Cl₂ (3 × 500 mL). The combined organic layer was dried over Na₂SO₄, filtered, and concentrated under reduced pressure, and the resulting crude residue was purified by column chromatography on silica gel (*R*_f = 0.58; 10% MeOH in CH₂Cl₂; gradient CH₂Cl₂/MeOH, 99:1, v/v; 49:1, v/v; 97:3, v/v) to give **10** (21.9 g, 75%) as a white foam. Mp 109 °C. ¹H NMR (500 MHz, CDCl₃) δ 9.38 (br s, 1H, NH-3), 8.08 (d, *J* = 8.1 Hz, 1H, H-6), 7.69–7.38 (m, 10H, Ar-H TBDPS), 5.60 (d, *J* = 8.1 Hz, 1H, H-5), 4.68 (br s, 1H, H-2'), 4.11 (d, *J* = 13.4 Hz, 1H, H-1'_{eq}),

3.99–3.94 (m, 2H, H-6' and H-4'), 3.89 (dd, $J = 11.0, 4.3$ Hz, 1H, H-6''), 3.83 (dd, $J = 13.5, 3.2$ Hz, 1H, H-1'_{ax}), 3.29–3.25 (m, 1H, H-5'), 2.51 (br s, 1H, OH-4'), 2.40–2.36 (m, 1H, H-3'_{eq}), 1.88–1.82 (m, 1H, H-3'_{ax}), 1.07 (s, 9H, 3 × CH₃-tBu); ¹³C NMR (125 MHz, CDCl₃) δ 163.3 (C-4), 151.0 (C-2), 142.9 (C-6), 135.5, 135.5 (Ar C-TBDPS), 132.7, 132.6 (*ipso* C-TBDPS), 130.1, 130.0, 127.9, 127.8 (Ar C-TBDPS), 102.0 (C-5), 81.2 (C-5'), 68.3 (C-1'), 63.9 (C-6'), 62.9 (C-4'), 51.1 (C-2'), 35.5 (C-3'), 26.8 (3 × CH₃-tBu), 19.2 (1C-tBu); HRMS (ESI-TOF) m/z : [M + H]⁺ calcd for C₂₆H₃₂N₂O₅Si, 481.2153; found, 481.2155.

1',5'-Anhydro-6'-O-tert-butylidiphenylsilyl-4'-O-mesyl-2',3'-dideoxy-2'-(uracil-1-yl)-D-alstro-hexitol (11). Mesyl chloride (8.5 mL, 109.7 mmol) was added slowly to a stirred solution of compound 10 (21.1 g, 43.9 mmol) in anhydrous pyridine (250 mL) at 0 °C, and the resulting reaction mixture was slowly warmed to room temperature over 4 h and stirred at the same temperature for 12 h. The reaction mixture was then cooled to –20 °C and neutralized with 5% NaHCO₃ solution. The aqueous layer was extracted with CH₂Cl₂ (3 × 400 mL). The combined organic layer was dried over Na₂SO₄, filtered, and concentrated under reduced pressure, and the resulting crude residue was purified by column chromatography on silica gel ($R_f = 0.68$; 10% MeOH in CH₂Cl₂; gradient CH₂Cl₂/MeOH, 199:1, v/v; 99:1, v/v; 19.7:0.3, v/v) to give 11 (24 g, 98%) as an off-white foam. Mp 88 °C. ¹H NMR (300 MHz, CDCl₃) δ 9.94 (br s, 1H, NH-3), 8.00 (d, $J = 8.1$ Hz, 1H, H-6), 7.71–7.36 (m, 10H, Ar-H TBDPS), 5.58 (d, $J = 8.1$ Hz, 1H, H-5), 5.10–5.02 (m, 1H, H-4'), 4.83–4.79 (m, 1H, H-2'), 4.15 (d, $J = 13.5$ Hz, 1H, H-1'_{eq}), 3.97 (dd, $J = 11.8, 2.2$ Hz, 1H, H-6'), 3.92–3.84 (m, 2H, H-6' and H-1'_{ax}), 3.51–3.46 (m, 1H, H-5'), 2.88 (s, 3H, CH₃-OMs), 2.70–2.62 (m, 1H, H-3'_{eq}), 2.24–2.14 (m, 1H, H-3'_{ax}), 1.09 (s, 9H, 3 × CH₃-tBu); ¹³C NMR (75 MHz, CDCl₃) δ 163.4 (C-4), 151.2 (C-2), 142.1 (C-6), 135.7 (Ar C-TBDPS), 132.8, 132.7 (*ipso* C-TBDPS), 130.1, 130.0, 127.9, 127.8 (Ar C-TBDPS), 102.9 (C-5), 79.2 (C-5'), 70.7 (C-4'), 68.4 (C-1'), 61.9 (C-6'), 50.3 (C-2'), 38.7 (CH₃-OMs), 33.9 (C-3'), 26.9 (3 × CH₃-tBu), 19.3 (1C-tBu); HRMS (ESI-TOF) m/z : [M + H]⁺ calcd for C₂₇H₃₄N₂O₇Si, 559.1929; found, 559.1919.

1',5'-Anhydro-4'-2-anhydro-6'-O-tert-butylidiphenylsilyl-2',3'-dideoxy-2'-(uracil-1-yl)-D-iodo-hexitol (12). Triethylamine (6.5 mL, 46.9 mmol) was added to a stirred solution of compound 11 (23.8 g, 42.6 mmol) in absolute ethanol (300 mL), and the resulting reaction mixture was heated at 90 °C for 16 h. The reaction mixture was then cooled, and volatiles were removed under reduced pressure. The crude residue was diluted with 5% NaHCO₃ solution (500 mL), and the aqueous layer was extracted with CH₂Cl₂ (3 × 400 mL). The combined organic layer was dried over Na₂SO₄, filtered, and concentrated under reduced pressure, and the resulting crude residue was purified by column chromatography on silica gel ($R_f = 0.35$; 10% MeOH in CH₂Cl₂; gradient CH₂Cl₂/MeOH, 99:1, v/v; 97:3, v/v; 47:3, v/v) to yield 12 (19.3 g, 98%) as a white foam. Mp 110 °C. ¹H NMR (300 MHz, CDCl₃) δ 7.67–7.28 (m, 11H, H-6 and Ar-H TBDPS), 5.98 (d, $J = 7.3$ Hz, 1H, H-5), 4.83 (br s, 1H, H-4'), 4.38 (br s, 1H, H-2'), 3.89 (d, $J = 12.1$ Hz, 1H, H-1'_{eq}), 3.81 (dd, $J = 8.8, 7.5$ Hz, 1H, H-6'), 3.77–3.63 (m, 3H, H-6', H-1'_{ax} and H-5'), 2.49–2.42 (m, 1H, H-3'_{eq}), 2.26–2.19 (m, 1H, H-3'_{ax}), 1.05 (s, 9H, 3 × CH₃-tBu); ¹³C NMR (75 MHz, CDCl₃) δ 171.7 (C-4), 155.9 (C-2), 141.2 (C-6), 135.5, 135.4 (Ar C-TBDPS), 133.1, 132.8 (*ipso* C-TBDPS), 129.9, 127.9, 127.8 (Ar C-TBDPS), 110.7 (C-5), 79.6 (C-5'), 70.0 (C-4'), 69.9 (C-1'), 62.1 (C-6'), 53.4 (C-2'), 26.8 (3 × CH₃-tBu), 26.4 (C-3'), 19.2 (1C-tBu); HRMS (ESI-TOF) m/z : [M + H]⁺ calcd for C₂₆H₃₀N₂O₄Si, 463.2047; found, 463.2044.

1',5'-Anhydro-6'-O-tert-butylidiphenylsilyl-4'-azido-2',3'-dideoxy-2'-(uracil-1-yl)-D-alstro-hexitol (13). To a stirred solution of compound 12 (19 g, 41.1 mmol) in dry DMF (300 mL) was added lithium azide (13.1 g, 267.1 mmol), and the resulting reaction mixture was heated at 135 °C for 48 h. The progress of reaction was monitored by TLC, and, after completion, the reaction mixture was cooled and volatiles were removed under reduced pressure. The crude residue was purified by column chromatography on silica gel to provide 13 ($R_f = 0.86$; 10% MeOH in CH₂Cl₂; gradient CH₂Cl₂/MeOH, 100:0, v/v; 199:1, v/v; 99:1, v/v; 3.95 g, 19%) as an off-white

foam and 14 ($R_f = 0.27$; 10% MeOH in CH₂Cl₂; gradient CH₂Cl₂/MeOH, 99:1, v/v; 49:1, v/v; 24:1, v/v; 3.95 g, 36%) as an off-white solid. Spectral data for 13: ¹H NMR (300 MHz, CDCl₃) δ 9.62 (br s, 1H, NH-3), 8.20 (d, $J = 8.2$ Hz, 1H, H-6), 7.72–7.36 (m, 10H, Ar-H TBDPS), 5.58 (d, $J = 8.1$ Hz, 1H, H-5), 4.73–4.69 (m, 1H, H-2'), 4.19 (dd, $J = 13.4, 1.8$ Hz, 1H, H-1'_{eq}), 3.99–3.83 (m, 4H, H-6', H-6'', H-4', and H-1'_{ax}), 3.24–3.19 (m, 1H, H-5'), 2.57–2.49 (m, 1H, H-3'_{eq}), 1.99–1.89 (m, 1H, H-3'_{ax}), 1.07 (s, 9H, 3 × CH₃-tBu); ¹³C NMR (75 MHz, CDCl₃) δ 163.4 (C-4), 151.3 (C-2), 142.9 (C-6), 135.7, 135.6 (Ar C-TBDPS), 133.0, 132.9 (*ipso* C-TBDPS), 130.1, 130.0, 127.9 (Ar C-TBDPS), 102.6 (C-5), 80.8 (C-5'), 68.5 (C-1'), 62.9 (C-6'), 51.7 (C-4'), 50.1 (C-2'), 33.3 (C-3'), 26.9 (3 × CH₃-tBu), 19.4 (1C-tBu); HRMS (ESI-TOF) m/z : [M + H]⁺ calcd for C₂₆H₃₁N₃O₄Si, 506.2218; found, 506.2208.

Spectral data for 14: ¹H NMR (500 MHz, DMSO-*d*₆) δ 11.26 (br s, 1H, NH-3), 8.00 (d, $J = 8.1$ Hz, 1H, H-6), 5.53 (d, $J = 8.1$ Hz, 1H, H-5), 4.86 (m, 1H, OH-6'), 4.48–4.46 (m, 1H, H-2'), 4.09–4.06 (m, 1H, H-1'_{eq}), 3.83–3.77 (m, 2H, H-4' and H-1'_{ax}), 3.65–3.55 (m, 2H, H-6' and H-6''), 3.25–3.21 (m, 1H, H-5'), 2.37–2.32 (m, 1H, H-3'_{eq}), 1.92–1.86 (m, 1H, H-3'_{ax}); ¹³C NMR (125 MHz, DMSO-*d*₆) δ 163.4 (C-4), 151.1 (C-2), 143.1 (C-6), 100.9 (C-5), 79.7 (C-5'), 67.0 (C-1'), 60.5 (C-6'), 52.4 (C-4'), 49.5 (C-2'), 31.4 (C-3'); HRMS (ESI-TOF) m/z : [M + H]⁺ calcd for C₁₀H₁₃N₃O₄, 268.1040; found, 268.1037.

Compound 13 was also synthesized (96%) starting from 14, following a procedure similar to that used for the synthesis of 10.

1',5'-Anhydro-4'-azido-2',3'-dideoxy-2'-(uracil-1-yl)-D-alstro-hexitol (14). To a stirred solution of compound 13 (0.9 g, 1.78 mmol) in dry THF (30 mL) in a plastic vessel was added triethylamine trihydrofluoride (1.16 mL, 7.12 mmol), and the resulting reaction mixture was heated at 50 °C for 60 h. The progress of the reaction was monitored by TLC, and after completion the reaction mixture was then cooled, and volatiles were removed under reduced pressure. Remaining hydrofluoride was quenched by addition of excess of silica gel, and the crude residue was purified by column chromatography on silica gel ($R_f = 0.27$; 10% MeOH in CH₂Cl₂; gradient CH₂Cl₂/MeOH, 99:1, v/v; 49:1, v/v; 24:1, v/v) to provide 14 (0.4 g, 85%) as an off-white solid. Mp 199 °C.

1',5'-Anhydro-6'-O-benzoyl-4'-azido-2',3'-dideoxy-2'-(uracil-1-yl)-D-alstro-hexitol (15). To a stirred solution of compound 14 (1.90 g, 7.11 mmol) in anhydrous pyridine (40 mL) was added benzoyl chloride (0.84 mL, 7.25 mmol) at 0 °C, and the resulting reaction mixture was slowly warmed to room temperature and stirred at the same temperature for 16 h. The reaction mixture was then cooled to 0 °C, and the pH was adjusted to ~8 with the addition of 5% NaHCO₃ solution. The aq layer was extracted with CH₂Cl₂ (3 × 200 mL). The combined organic layer was dried over Na₂SO₄, filtered, and concentrated under reduced pressure, and the resulting crude residue was purified by column chromatography on silica gel ($R_f = 0.63$; 10% MeOH in CH₂Cl₂; gradient CH₂Cl₂/MeOH, 199:1, v/v; 99:1, v/v; 66:1, v/v) to give 15 (2.37 g, 90%) as a white semisolid. Mp 91 °C. ¹H NMR (300 MHz, CDCl₃) δ 9.76 (br s, 1H, NH-3), 8.07–8.01 (m, 3H, H-6 and *o*-H Bz), 7.61 (t, 1H, $J = 7.4$ Hz, *p*-H Bz), 7.47 (t, 2H, $J = 7.7$ Hz, *m*-H Bz), 5.52 (d, $J = 8.2$ Hz, 1H, H-5), 4.75–4.68 (m, 2H, H-6' and H-2'), 4.52 (dd, $J = 12.3, 3.6$ Hz, 1H, H-6''), 4.25 (d, $J = 13.9$ Hz, 1H, H-1'_{eq}), 3.95 (dd, $J = 13.7, 3.2$ Hz, 1H, H-1'_{ax}), 3.77–3.69 (m, 1H, H-4'), 3.59–3.54 (m, 1H, H-5'), 2.66–2.58 (m, 1H, H-3'_{eq}), 2.06–1.96 (m, 1H, H-3'_{ax}); ¹³C NMR (75 MHz, CDCl₃) δ 166.0 (CO-Bz), 163.3 (C-4), 151.2 (C-2), 142.4 (C-6), 133.7 (*p*C-Bz), 129.6 (*o*C-Bz), 129.5 (*ipso* C-Bz), 128.7 (*m*C-Bz), 102.4 (C-5), 78.4 (C-5'), 68.3 (C-1'), 63.3 (C-6'), 52.4 (C-4'), 50.3 (C-2'), 33.0 (C-3'); HRMS (ESI-TOF) m/z : [M + H]⁺ calcd for C₁₇H₁₇N₃O₅, 372.1302; found, 372.1318.

1',5'-Anhydro-6'-O-benzoyl-4'-amino-2',3'-dideoxy-2'-(uracil-1-yl)-D-alstro-hexitol (16). To a stirred solution of 15 (2.3 g, 6.19 mmol, 1 equiv) in absolute EtOH (130 mL, degassed with argon) were added two drops of glacial AcOH followed by 10% Pd/C (Degussa type, 0.345 g, 0.15 equiv w/w), and evacuation was then carried out with hydrogen atmosphere replacements (3×). The reaction mixture was stirred at room temperature for 16 h under an

atmospheric pressure of hydrogen. After completion of the reaction, the catalyst was removed by filtration through a Celite pad, and the pad was thoroughly washed with EtOH. The combined filtrate was concentrated under reduced pressure, and the crude residue was purified by column chromatography on silica gel ($R_f = 0.29$; 10% MeOH in CH_2Cl_2 ; gradient $\text{CH}_2\text{Cl}_2/\text{MeOH}$, 99:1, v/v; 24:1, v/v; 93:7, v/v) to afford **16** (1.94 g, 91%) as a white semisolid. Mp 128 °C. ^1H NMR (300 MHz, CDCl_3) δ 8.14 (d, $J = 8.1$ Hz, 1H, H-6), 8.04–8.02 (m, 2H, *o*-H Bz), 7.63–7.57 (m, 1H, *p*-H Bz), 7.46 (t, 2H, $J = 7.8$ Hz, *m*-H Bz), 5.46 (d, $J = 8.1$ Hz, 1H, H-5), 4.79 (dd, 1H, $J = 12.2$, 3.3 Hz, H-6'), 4.61 (br s, 1H, H-2'), 4.56 (dd, $J = 12.2$, 2.2 Hz, 1H, H-6'), 4.21 (dd, $J = 13.4$, 1.4 Hz, 1H, H-1'eq), 3.93 (dd, $J = 13.6$, 3.2 Hz, 1H, H-1'ax), 3.46–3.41 (m, 1H, H-5'), 3.06–2.98 (m, 1H, H-4'), 2.35–2.27 (m, 1H, H-3'eq), 1.81–1.71 (m, 1H, H-3'ax); ^{13}C NMR (75 MHz, CDCl_3) δ 166.5 (CO-Bz), 163.6 (C-4), 151.3 (C-2), 143.1 (C-6), 133.6 (*p*C-Bz), 129.7 (*o*C-Bz), 129.6 (*ipso* C-Bz), 128.6 (*m*C-Bz), 101.8 (C-5), 82.0 (C-5'), 68.4 (C-1'), 63.5 (C-6'), 50.8 (C-2'), 42.7 (C-4'), 36.5 (C-3'); HRMS (ESI-TOF) m/z : $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{17}\text{H}_{19}\text{N}_3\text{O}_5$, 346.1397; found, 346.1402.

1',5'-Anhydro-6'-O-benzoyl-4'-amino(monomethoxytrityl)-2',3'-dideoxy-2'-(uracil-1-yl)-D-althro-hexitol (17). To a stirred solution of compound **16** (1.9 g, 5.5 mmol) in dry pyridine (60 mL) were added *N,N*-diisopropylethylamine (9.6 mL, 55 mmol) followed by 4-methoxytrityl chloride (5.09 g, 16.5 mmol), and the resulting reaction mixture was stirred at room temperature for 24 h. The progress of the reaction was monitored by TLC, and, after completion, the reaction mixture was quenched with 5% NaHCO_3 solution. The aq layer was extracted with CH_2Cl_2 (3 $\times$ 200 mL). The combined organic layer was dried over Na_2SO_4 , filtered, and concentrated under reduced pressure, and the resulting crude residue was purified by column chromatography on silica gel ($R_f = 0.52$; 80% EtOAc in hexane; gradient hexane/EtOAc, 4:1, v/v; 3:2, v/v; 2:3, v/v) to give **17** (3.02 g, 89%) as a white solid. ^1H NMR (600 MHz, CDCl_3) δ 7.83–7.81 (m, 2H, *o*H-Bz), 7.75 (d, $J = 8.1$ Hz, 1H, H-6), 7.61–7.58 (m, 1H, *p*H-Bz), 7.45–6.68 (m, 16H, Ar-H MMTTr and *m*H-Bz), 5.33 (d, $J = 8.1$ Hz, 1H, H-5), 4.70 (dd, $J = 12.1$, 3.5 Hz, 1H, H-6'), 4.58 (dd, $J = 12.0$, 2.7 Hz, 1H, H-6'), 4.36–4.34 (m, 1H, H-2'), 4.01–3.98 (m, 1H, H-1'eq), 3.86 (dd, $J = 13.3$, 3.5 Hz, H-1'ax), 3.70 (s, 3H, OCH_3), 3.66–3.64 (m, 1H, H-5'), 2.75–2.70 (m, 1H, H-4'), 1.97 (d, $J = 6.5$ Hz, 1H, NH-4'), 1.61–1.57 (m, 1H, H-3'eq), 1.53–1.48 (m, 1H, H-3'ax); ^{13}C NMR (150 MHz, CDCl_3) δ 166.1 (CO-Bz), 162.6 (C-4), 158.1 (*p*C-anisyl), 150.2 (C-2), 146.4, 146.3 (*ipso* C-Ph), 142.8 (C-6), 137.7 (*ipso* C-anisyl), 133.3, 129.8, 129.6, 128.4, 128.4, 128.3, 127.9, 126.6, 126.5 (Ar C-anisyl and Ph), 113.2 (*m*C-anisyl), 101.2 (C-5), 79.9 (C-5'), 70.0 (1C-MMTTr), 67.9 (C-1'), 63.3 (C-6'), 55.1 (OCH_3 -anisyl), 50.3 (C-2'), 46.4 (C-4'), 35.0 (C-3'); HRMS (ESI-TOF) m/z : $[\text{M} + \text{Na}]^+$ calcd for $\text{C}_{37}\text{H}_{35}\text{N}_3\text{O}_6$, 640.2418; found, 640.2412.

1',5'-Anhydro-4'-amino(monomethoxytrityl)-2',3'-dideoxy-2'-(uracil-1-yl)-D-althro-hexitol (1). A stirred solution of compound **17** (2.63 g, 4.26 mmol) in 7 N NH_3 in MeOH (30 mL) in a sealed vessel was heated at 55 °C for 48 h. After completion, the reaction mixture was cooled, and volatiles were removed under reduced pressure. The resulting crude residue was purified by column chromatography on silica gel ($R_f = 0.28$; EtOAc; gradient hexane/EtOAc, 3:2, v/v; 2:3, v/v; 0:100, v/v) to afford **1** (1.86 g, 85%) as a white semisolid. ^1H NMR (500 MHz, $\text{DMSO}-d_6$) δ 11.2 (br s, 1H, NH-3), 7.55 (d, $J = 8.1$ Hz, 1H, H-6), 7.44–6.77 (m, 14H, Ar-H Trt), 5.34 (d, $J = 8.0$ Hz, 1H, H-5), 4.72 (t, $J = 5.5$ Hz, 1H, OH-6'), 4.20–4.17 (m, 1H, H-2') 3.86 (d, $J = 13.2$ Hz, 1H, H-1'eq), 3.71–3.68 (m, 4H, OCH_3 and H-1'ax), 3.52–3.38 (m, 2H, H-6' and H-6''), 3.30–3.27 (m, 1H, H-5'), 3.09 (d, $J = 5.6$ Hz, 1H, NH-4'), 2.23–2.17 (m, 1H, H-4'), 1.65–1.61 (m, 1H, H-3'eq), 1.54–1.49 (m, 1H, H-3'ax); ^{13}C NMR (125 MHz, $\text{DMSO}-d_6$) δ 163.2 (C-4), 157.5 (*p*C-anisyl), 150.7 (C-2), 147.3, 147.2 (*ipso* C-Ph), 142.8 (C-6), 138.2 (*ipso* C-anisyl), 129.7, 128.9, 128.3, 128.2, 127.6, 126.1, 125.4 (Ar C-anisyl and Ph), 112.9 (*m*C-anisyl), 100.5 (C-5), 80.2 (C-5'), 69.7 (1C-MMTTr), 66.2 (C-1'), 62.3 (C-6'), 54.9 (OCH_3 -anisyl), 49.8 (C-2'), 48.7 (C-4'), 33.4 (C-3'); HRMS (ESI-TOF) m/z : $[\text{M} + \text{Na}]^+$ calcd for $\text{C}_{30}\text{H}_{31}\text{N}_3\text{O}_5$, 536.2156; found, 536.2137.

1',5'-Anhydro-6'-O-tert-butylidiphenylsilyl-4'-azido-2',3'-dideoxy-2'-(cytosine-1-yl)-D-althro-hexitol (18). To a stirred suspension of 1,2,4-triazole (21.04 g, 304.6 mmol) in dry ACN (400 mL) was added POCl_3 dropwise (6.6 mL, 69.6 mmol) at 0 °C. After being stirred for 15 min at the same temperature, dry triethylamine (48.4 mL, 348 mmol) was added to the reaction mixture, and stirring was continued for an additional 30 min. Nucleoside **13** (4.4 g, 8.70 mmol) dissolved in dry ACN (100 mL) was then added to the reaction mixture and stirred at 35 °C for 13 h. After completion, the reaction mixture was diluted with ethyl acetate, and organic layer was washed with water followed by 5% NaHCO_3 solution and brine. Organic layer was dried over Na_2SO_4 , filtered, and concentrated under reduced pressure to give crude triazolidine, which was used for the next reaction without purification.

Crude triazolidine was dissolved in 1,4 dioxane (100 mL), 25% aq ammonia solution (40 mL) was added to the solution, and the reaction mixture was stirred at 35 °C for 16 h. The reaction mixture was concentrated under reduced pressure, coevaporated with toluene (2 $\times$), and crude residue was purified by column chromatography on silica gel ($R_f = 0.28$; 10% MeOH in CH_2Cl_2 ; gradient $\text{CH}_2\text{Cl}_2/\text{MeOH}$, 99:1, v/v; 49:1, v/v; 24:1, v/v) to give **18** (4.19 g, 95% over two steps) as a pale yellow semisolid. Mp 118 °C. ^1H NMR (500 MHz, $\text{DMSO}-d_6$) δ 8.56 (br s, 1H, NH-4), 8.03 (d, $J = 7.4$ Hz, 1H, H-6), 7.69–7.42 (m, 10H, Ar-H TBDPS), 7.08 (br s, 1H, NH-4), 5.59 (d, $J = 7.4$ Hz, 1H, H-5), 4.57–4.54 (m, 1H, H-2'), 4.15 (d, $J = 13.3$ Hz, 1H, H-1'eq), 3.92–3.79 (m, 4H, H-1'ax, H-4', H-6', and H-6''), 3.36–3.34 (m, 1H, H-5'), 2.43–2.37 (m, 1H, H-3'eq), 1.99–1.91 (m, 1H, H-3'ax), 1.01 (s, 9H, 3 $\times$ CH_3 -Bu); ^{13}C NMR (125 MHz, $\text{DMSO}-d_6$) δ 165.5 (C-4), 155.5 (C-2), 143.3 (C-6), 135.2, 135.1 (Ar C-Ph), 132.7, 132.6 (*ipso* C-Ph), 130.0, 129.9, 127.9 (Ar C-Ph), 93.2 (C-5), 79.3 (C-5'), 67.9 (C-1'), 63.0 (C-6'), 52.2 (C-4'), 49.8 (C-2'), 31.7 (C-3'), 26.6 (3 $\times$ CH_3 -Bu), 18.9 (1C-Bu); HRMS (ESI-TOF) m/z : $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{26}\text{H}_{32}\text{N}_6\text{O}_3\text{Si}$, 505.2378; found, 505.2354.

1',5'-Anhydro-6'-O-tert-butylidiphenylsilyl-4'-azido-2',3'-dideoxy-2'-(N⁴-benzoyl-cytosine-1-yl)-D-althro-hexitol (19). To a stirred solution of compound **18** (4.19 g, 8.30 mmol) in anhydrous pyridine (70 mL) was added benzoyl chloride (1.63 mL, 14.11 mmol) at 0 °C, and the resulting reaction mixture was slowly warmed to room temperature and stirred at the same temperature for 30 h. The reaction mixture was then cooled to 0 °C, and the pH was adjusted to ~8 with the addition of 5% NaHCO_3 solution. The aq layer was extracted with CH_2Cl_2 (3 $\times$ 150 mL), 25% aq ammonia (30 mL) was added at 0 °C to the organic phase, and the mixture was stirred for 1 h. The reaction mixture was concentrated under reduced pressure, coevaporated with toluene (2 $\times$), and crude residue was purified by column chromatography on silica gel ($R_f = 0.51$; EtOAc; gradient hexane/EtOAc, 3:2, v/v; 2:3, v/v; 0:100, v/v) to give **19** (3.7 g, 75% over two steps) as a white foam. Mp 91 °C. ^1H NMR (500 MHz, CDCl_3) δ 8.61 (d, $J = 7.5$ Hz, 1H, H-6), 7.91 (d, $J = 7.8$ Hz, 2H, *o*H-Bz), 7.74–7.68 (m, 4H, Ar-H TBDPS), 7.62 (t, $J = 7.5$ Hz, 1H, *p*H-Bz), 7.52 (d, $J = 7.8$ Hz, 2H, *m*H-Bz), 7.47–7.39 (m, 7H, Ar-H TBDPS and H-5), 4.89–4.87 (m, 1H, H-2'), 4.27 (dd, $J = 13.4$, 1.8 Hz, 1H, H-1'eq), 3.96–3.86 (m, 4H, H-1'ax, H-4', H-6', and H-6''), 3.26–3.24 (m, 1H, H-5'), 2.70–2.65 (m, 1H, H-3'eq), 2.03–1.97 (m, 1H, H-3'ax), 1.72 (br s, 1H, NH-4), 1.10 (s, 9H, 3 $\times$ CH_3 -Bu); ^{13}C NMR (125 MHz, CDCl_3) δ 163.6 (C-4), 161.9 (CO-Bz), 159.7 (C-2), 147.6 (C-6), 135.6, 135.6, 133.2, 132.9, 132.8, 129.9, 129.0, 127.8, 127.7, 127.5 (Ar C-Bz and TBDPS), 96.9 (C-5), 80.8 (C-5'), 68.5 (C-1'), 62.8 (C-6'), 51.6 (C-4'), 51.4 (C-2'), 32.4 (C-3'), 26.8 (3 $\times$ CH_3 -Bu), 19.3 (1C-Bu); HRMS (ESI-TOF) m/z : $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{33}\text{H}_{36}\text{N}_6\text{O}_4\text{Si}$, 609.2640; found, 609.2641.

1',5'-Anhydro-6'-O-tert-butylidiphenylsilyl-4'-amino-2',3'-dideoxy-2'-(N⁴-benzoyl-cytosine-1-yl)-D-althro-hexitol (20). A synthetic protocol similar to that used for the synthesis of **16** was employed for the synthesis of **20**, starting from **19** (3.7 g, 6.08 mmol), 10% Pd/C (0.74 g, 0.2 equiv w/w), and EtOH (250 mL) to obtain **20** (2.96 g, 84%) as a white foam ($R_f = 0.26$, 10% MeOH in CH_2Cl_2 ; column chromatography gradient $\text{CH}_2\text{Cl}_2/\text{MeOH}$, 99:1, v/v; 49:1, v/v; 24:1, v/v). ^1H NMR (300 MHz, CDCl_3) δ 8.66 (d, $J = 7.4$ Hz, 1H,

H-6), 8.63–8.60 (m, 1H, NH-4), 7.91 (d, $J = 7.4$ Hz, 2H, oH-Bz), 7.72–7.67 (m, 4H, Ar-H TBDPS), 7.62–7.59 (m, 1H, pH-Bz), 7.53 (d, $J = 7.8$ Hz, 2H, mH-Bz), 7.47–7.37 (m, 7H, Ar-H TBDPS and H-5), 4.81 (br s, 1H, H-2'), 4.25 (dd, $J = 13.6, 1.9$ Hz, 1H, H-1'eq), 3.96–3.87 (m, 3H, H-1'ax, H-6', and H-6''), 3.21–3.09 (m, 2H, H-4' and H-5'), 2.43–2.35 (m, 1H, H-3'eq), 1.79–1.49 (m, 3H, H-3'ax and NH₂-4'), 1.10 (s, 9H, $3 \times \text{CH}_3$ -tBu); ¹³C NMR (75 MHz, CDCl₃) δ 163.1 (C-4), 161.9 (CO-Bz), 159.7 (C-2), 148.3 (C-6), 135.8, 135.7, 133.3, 133.2, 130.1, 130.0, 129.2, 128.0, 127.9, 127.7, 123.8 (Ar C-Bz and TBDPS), 96.6 (C-5), 84.4 (C-5'), 68.8 (C-1'), 63.5 (C-6'), 52.2 (C-2'), 42.4 (C-4'), 35.9 (C-3'), 27.0 ($3 \times \text{CH}_3$ -tBu), 19.5 (1C-tBu); HRMS (ESI-TOF) m/z : [M + H]⁺ calcd for C₃₃H₃₈N₄O₄Si, 583.2735; found, 583.2747.

1',5'-Anhydro-6'-O-tert-butylidiphenylsilyl-4'-amino(trityl)-2',3'-dideoxy-2'-(N⁴-benzoyl-cytosine-1-yl)-D-alto-hexitol (21). A slightly modified synthetic protocol from that used for the synthesis of 17 was employed for the synthesis of 21, starting from 20 (2.5 g, 4.29 mmol), trityl chloride (4.78 g, 17.16 mmol), and *N,N*-diisopropylethylamine (8.97 mL, 51.48 mmol) in dry pyridine (80 mL) at 55 °C for 120 h to obtain 20 (3.11 g, 88%) as a light brown semisolid ($R_f = 0.65$, EtOAc; column chromatography gradient hexane/EtOAc, 3:2, v/v; 2:3, v/v; 0:100, v/v). Mp 128 °C. ¹H NMR (300 MHz, CDCl₃) δ 8.75 (br s, 1H, NH-4), 7.97–7.91 (m, 3H, H-6 and oH-Bz), 7.63–7.07 (m, 29H, Ar-H TBDPS, Trt, Bz and H-5), 4.59 (br s, 1H, H-2'), 3.93–3.76 (m, 4H, H-1'eq, H-1'ax, H-6', and H-6''), 3.44–3.39 (m, 1H, H-5'), 2.63–2.54 (m, 1H, H-4'), 2.33 (br s, 1H, NH-4'), 1.85–1.76 (m, 1H, H-3'eq), 1.70–1.60 (m, 1H, H-3'ax), 1.01 (s, 9H, $3 \times \text{CH}_3$ -tBu); ¹³C NMR (75 MHz, CDCl₃) δ 166.8 (C-4), 161.5 (CO-Bz), 158.5 (C-2), 147.4 (C-6), 135.8, 135.6, 133.2, 133.2, 133.0, 129.9, 129.8, 129.2, 128.7, 128.2, 128.1, 128.0, 127.9, 127.7, 126.5 (Ar C-Bz, Trt and TBDPS), 96.4 (C-5), 82.0 (C-5'), 70.6 (1C-Trt), 68.2 (C-1'), 64.5 (C-6'), 51.3 (C-2'), 48.0 (C-4'), 33.6 (C-3'), 27.0 ($3 \times \text{CH}_3$ -tBu), 19.3 (1C-tBu); HRMS (ESI-TOF) m/z : [M + H]⁺ calcd for C₅₂H₅₂N₄O₄Si, 825.3830; found, 825.3829.

1',5'-Anhydro-4'-amino(trityl)-2',3'-dideoxy-2'-(N⁴-benzoyl-cytosine-1-yl)-D-alto-hexitol (2). To a stirred solution of 21 (3.11 g, 3.77 mmol) in dry THF (90 mL) in a plastic vessel was added triethylamine trihydrofluoride (2.46 mL, 15.08 mmol), and the resulting reaction mixture was heated at 37 °C for 144 h. The progress of the reaction was monitored by TLC, and after completion the reaction mixture was then cooled and volatiles were removed under reduced pressure. Crude was diluted with ethyl acetate (200 mL), and the remaining hydrofluoride was quenched by addition of 5% NaHCO₃ solution. The aq layer was extracted with ethyl acetate (2 $\times$ 100 mL), the combined organic layer was dried over Na₂SO₄, filtered, and concentrated under reduced pressure, and the crude residue was purified by column chromatography on silica gel ($R_f = 0.29$, EtOAc; column chromatography gradient hexane/EtOAc, 3:2, v/v; 2:3, v/v; 0:100, v/v) to provide 2 (0.4 g, 85%) as a pale yellow semisolid. Mp 214 °C. ¹H NMR (600 MHz, DMSO-*d*₆) δ 11.19 (br s, 1H, NH-4), 8.07–8.06 (m, 2H, oH-Bz), 8.01 (d, $J = 7.0$ Hz, 1H, H-6), 7.66–7.63 (m, 1H, pH-Bz), 7.54 (t, $J = 8.0$ Hz, 2H, mH-Bz), 7.42–7.08 (m, 16H, Ar-H Trt and H-5), 4.74 (t, $J = 5.3$ Hz, 1H, OH-6'), 4.40 (br s, 1H, H-2'), 3.95 (d, $J = 12.6$ Hz, 1H, H-1'eq), 3.77 (dd, $J = 12.8, 3.6$ Hz, 1H, H-1'ax), 3.57–3.54 (m, 1H, H-6'), 3.42–3.39 (m, 1H, H-6''), 3.33–3.31 (m, 1H, H-5'), 3.16 (d, $J = 5.3$ Hz, 1H, NH-4'), 2.21–2.17 (m, 1H, H-4'), 1.77–1.73 (m, 1H, H-3'eq), 1.64–1.59 (m, 1H, H-3'ax), 1.01 (s, 9H, $3 \times \text{CH}_3$ -tBu); ¹³C NMR (150 MHz, DMSO-*d*₆) δ 167.2 (CO-Bz), 162.4 (C-4), 154.8 (C-2), 149.7 (Ar-C), 147.6 (C-6), 146.8, 132.8, 128.6, 128.5, 128.4, 127.7, 126.2, 124.0 (Ar C-Bz and Trt), 95.7 (C-5), 80.1 (C-5'), 70.2 (1C-Trt), 66.3 (C-1'), 59.8 (C-6'), 51.1 (C-2'), 48.5 (C-4'), 32.6 (C-3'); HRMS (ESI-TOF) m/z : [M + H]⁺ calcd for C₃₆H₃₄N₄O₄, 587.2653; found, 587.2673.

(2R,3S)-3-(Benzyloxy)-2-((benzyloxy)methyl)-3,6-dihydro-2H-pyran (25). A solution of diol 22 (66 g, 0.509 mol) in dry DMF (400 mL) was added dropwise to a stirred suspension of NaH (55% in mineral oil, 48.8 g, 1.12 mol) in dry DMF (400 mL) at –20 °C under argon, and the resulting mixture was stirred at room

temperature for 1 h until the H₂ gas liberation was complete. The reaction mixture was again cooled to –20 °C, and benzyl bromide (225 mL, 1.88 mol) was added slowly to the mixture. The reaction mixture then was slowly warmed to room temperature and left stirring for 16 h at same temperature. Upon completion, the reaction mixture was cooled to 0 °C and quenched with saturated NH₄Cl solution and diluted with water (8 L), and the aq layer was extracted with EtOAc (3 $\times$ 1 L). The combined organic layer was dried over Na₂SO₄, filtered and concentrated in vacuo, and the resulting crude residue was purified by column chromatography on silica gel ($R_f = 0.49$, 40% EtOAc in hexane; gradient hexane/EtOAc, 19:1, v/v; 9:1, v/v; 17:3, v/v) to give 25 (129 g, 90%) as a pale-yellow liquid. ¹H NMR (300 MHz, CDCl₃) δ 7.32–7.24 (m, 10H, 2 $\times$ Ar-H Bn), 6.05–5.95 (m, 2H, H-2' and H-3'), 4.67–4.52 (m, 4H, 2 $\times$ CH₂-Bn), 4.35–4.28 (m, 1H, H-1'eq), 4.19–4.13 (m, 1H, H-1'ax), 3.82–3.72 (m, 4H, H-4', H-5', H-6', and H-6''); ¹³C NMR (75 MHz, CDCl₃) δ 138.7, 138.3 (2 $\times$ ipso C-Bn), 131.6 (C-3'), 128.5, 128.4, 128.0, 127.9, 127.9, 127.8, 127.7, 127.6 (Ar C-Bn), 123.7 (C-2'), 76.8 (C-5'), 73.7 (CH₂-6'Bn), 70.7 (CH₂-4'Bn), 70.0 (C-4'), 68.7 (C-6'), 65.9 (C-1'); HRMS (ESI-TOF) m/z : [M + Na]⁺ calcd for C₂₀H₂₂O₃, 333.1461; found, 333.1462.

(1S,4R,5S,6S)-5-(Benzyloxy)-4-((benzyloxy)methyl)-3,7-dioxabicyclo[4.1.0]heptane (26). To a stirred solution of 25 (64.4 g, 207.5 mmol) in CH₂Cl₂ (900 mL) was added mCPBA (70% in water, 127.9 g, 518.7 mmol) at –40 °C under argon. The reaction mixture then was slowly warmed to room temperature and left stirring for 36 h at same temperature. Upon completion, the reaction mixture was filtered over sodium sulfate to remove water, and white precipitate was washed with cold CH₂Cl₂. Combined filtrate was concentrated in vacuo (bath temp 15 °C), and the resulting crude was repeatedly triturated with hexane and filtered to remove white precipitate. Combined filtrate was concentrated in vacuo, and the resulting crude residue was purified by column chromatography on silica gel ($R_f = 0.42$, 60% EtOAc in hexane; gradient hexane/EtOAc, 19:1, v/v; 9:1, v/v; 17:3, v/v) to give 26 (39.9 g, 59%) as a colorless liquid. ¹H NMR (300 MHz, CDCl₃) δ 7.34–7.28 (m, 10H, 2 $\times$ Ar-H Bn), 4.65 (dd, $J = 12.9, 11.7$ Hz, 2H, CH₂-6'Bn), 4.51 (dd, $J = 28.9, 12.1$ Hz, 2H, CH₂-4'Bn), 4.22 (dd, $J = 13.6, 3.4$ Hz, 1H, H-1'eq), 3.93 (d, $J = 13.7$ Hz, 1H, H-1'ax), 3.83 (br s, 1H, H-4'), 3.68–3.64 (m, 1H, H-5'), 3.63–3.47 (m, 2H, H-6' and H-6''), 3.32–3.28 (m, 2H, H-2' and H-3'); ¹³C NMR (75 MHz, CDCl₃) δ 128.6, 128.5, 128.4, 128.2, 127.9, 127.8 (Ar C-Bn), 73.6 (CH₂-6'Bn), 73.5 (CH₂-4'Bn), 70.2 (C-5'), 71.1 (C-4'), 69.3 (C-6'), 64.9 (C-1'), 51.8 (C-3'), 57.7 (C-2'); HRMS (ESI-TOF) m/z : [M + Na]⁺ calcd for C₂₀H₂₂O₄, 349.1410; found, 349.1406.

1',5'-Anhydro-4',6'-di-O-benzyl-2'-deoxy-2'-(adenin-9-yl)-D-allo-hexitol (27). To a stirred solution of 26 (39.2 g, 120.1 mmol) and adenine (34.1 g, 252.2 mmol) in dry DMF (450 mL) was added DBU (44.9 mL, 300.3 mmol) under argon, and the reaction mixture was heated at 105 °C for 20 h. The reaction was monitored with TLC; upon completion, the reaction mixture was cooled, and the volatiles were removed under reduced pressure. The residue was diluted with ice–water (900 mL) and neutralized with 1 N HCl at 0 °C. The aq layer was extracted with CH₂Cl₂ (3 $\times$ 700 mL). The combined organic layer was dried over Na₂SO₄, filtered, and concentrated in vacuo to provide a crude residue of an isomeric mixture of N⁷ (28) and N⁹ (27) adduct, which was used for further reaction without any purification.

The mixture was dissolved in dry pyridine (500 mL), and acetic anhydride (113.6 mL, 1.20 mol) was added at 0 °C to the reaction mixture and stirred at room temperature for 24 h. The reaction mixture was concentrated under reduced pressure, coevaporated with toluene (2 $\times$), and crude residue was purified by column chromatography on silica gel (column chromatography gradient CH₂Cl₂/MeOH, 199:1, v/v; 99:1, v/v; 66:1, v/v) to give di- and triacetylated adducts ($R_f = 0.74$ and 0.54, respectively, 10% MeOH in CH₂Cl₂) of 27. The di- and triacetylated adducts of 27 were combined and treated with 7 N NH₃ in MeOH for 24 h at room temperature. Volatiles were removed under reduced pressure, and crude residue was purified by column chromatography on silica gel (R_f

= 0.35, 10% MeOH in CH₂Cl₂; gradient CH₂Cl₂/MeOH, 99:1, v/v; 49:1, v/v; 24:1, v/v) to give **27** (37.7 g, 68% over three steps) as a white semisolid. ¹H NMR (300 MHz, CDCl₃) δ 8.29 (s, 1H, H-8), 8.20 (s, 1H, H-2), 7.34–6.83 (m, 10H, 2 × Ar-H Bn), 6.27 (br s, 2H, NH₂-6), 4.65–4.50 (m, 3H, CH₂-6'Bn and H-2'), 4.37–4.12 (m, 6H, H-1'eq, H-1'ax, H-5', H-3', and CH₂-4'Bn), 3.88 (dd, *J* = 10.1, 7.3 Hz, 1H, H-6'), 3.63 (dd, *J* = 10.2, 4.6 Hz, 1H, H-6''), 3.53 (t, *J* = 3.2 Hz, 1H, H-4'); ¹³C NMR (75 MHz, CDCl₃) δ 155.6 (C-6), 152.4 (C-2), 149.8 (C-4), 141.7 (C-8), 138.1, 137.4 (2 × ipso C-Bn), 128.6, 128.3, 128.0, 128.0, 127.9 (Ar C-Bn), 118.9 (C-5), 76.7 (C-4'), 74.4 (C-5'), 73.7 (CH₂-6'Bn), 73.1 (CH₂-4'Bn), 69.3 (C-6'), 65.2 (C-3'), 64.8 (C-1'), 54.2 (C-2'); HRMS (ESI-TOF) *m/z*: [M + H]⁺ calcd for C₂₅H₂₇N₅O₄, 462.2136; found, 462.2139.

1',5'-Anhydro-4',6'-di-O-benzyl-2',3'-dideoxy-2'-(adenin-9-yl)-D-allo-hexitol (30). A slightly modified synthetic protocol from that used for the synthesis of **7** was employed for the synthesis of **29**, starting from **27** (37.7 g, 81.69 mmol), aq NaOH (5N; 89.9 mL), CS₂ (89.9 mL), and ethyl bromide (26.1 mL) in DMF (800 mL) in the first step to obtain crude xanthate **29** (46.2 g, quant), which was used for the next step without further purification. Workup was done using ice-cold water, and organic solvents were removed in vacuo keeping the bath temp below 12 °C.

A Barton–McCombie condition similar to that used for the synthesis of **8** was employed for the synthesis of **30**, starting from crude **29** (46.2 g), *n*Bu₃SnH (48.4 mL, 179.7 mmol), and AIBN (2.01 g, 12.2 mmol) in anhydrous toluene (800 mL) to obtain **30** (21.84 g, 60% over two steps) as a white solid (*R*_f = 0.37, 10% MeOH in CH₂Cl₂; column chromatography gradient CH₂Cl₂/MeOH, 99:1, v/v; 49:1, v/v; 97:3, v/v). Mp 148 °C. ¹H NMR (600 MHz, CDCl₃) δ 8.52 (s, 1H, H-8), 8.34 (s, 1H, H-2), 7.35–6.87 (m, 10H, 2 × Ar-H Bn), 6.28 (br s, 2H, NH₂-6), 4.70–4.69 (m, 1H, H-2'), 4.56 (dd, *J* = 73.6, 11.9 Hz, 2H, CH₂-6'Bn), 4.43–4.40 (m, 1H, H-1'eq), 4.07 (dd, *J* = 13.0, 3.0 Hz, 1H, H-1'ax), 4.01 (s, 2H, CH₂-4'Bn), 3.83–3.79 (m, 2H, H-5' and H-6'), 3.63 (dd, *J* = 9.0, 4.1 Hz, 1H, H-6''), 3.61–3.59 (m, 1H, H-4'), 2.64–2.61 (m, 1H, H-3'eq), 2.00–1.96 (m, 1H, H-3'ax); ¹³C NMR (150 MHz, CDCl₃) δ 155.5 (C-6), 152.4 (C-2), 149.7 (C-4), 141.5 (C-8), 137.8, 137.2 (2 × ipso C-Bn), 128.3, 128.1, 127.7, 127.7, 127.6, 127.5 (Ar C-Bn), 118.7 (C-5), 78.9 (C-5'), 73.5 (CH₂-6'Bn), 70.9 (CH₂-4'Bn), 70.4 (C-4'), 70.0 (C-6'), 69.7 (C-1'), 46.8 (C-2'), 29.4 (C-3'); HRMS (ESI-TOF) *m/z*: [M + H]⁺ calcd for C₂₅H₂₇N₅O₃, 446.2186; found, 446.2181.

1',5'-Anhydro-4',6'-di-O-benzyl-2',3'-dideoxy-2'-(N⁶-benzoyladenin-9-yl)-D-allo-hexitol (31). A synthetic protocol similar to that used for the synthesis of **19** was employed for the synthesis of **31**, starting from **30** (21.8 g, 48.93 mmol) and benzoyl chloride (12.5 mL, 107.64 mmol) in anhydrous pyridine (250 mL) to obtain **31** (25.5 g, 95%) as a white sticky mass (*R*_f = 0.6, 10% MeOH in CH₂Cl₂; column chromatography gradient CH₂Cl₂/MeOH, 100:0, v/v; 99:1, v/v; 49:1, v/v). ¹H NMR (600 MHz, CDCl₃) δ 9.18 (br s, 1H, NH-6), 8.81 (s, 1H, H-2), 8.71 (s, 1H, H-8), 8.05–6.82 (m, 15H, 2 × Ar-H Bn and Bz), 4.78 (br s, 1H, H-2'), 4.57 (dd, *J* = 72.9, 11.9 Hz, 2H, CH₂-6'Bn), 4.48–4.46 (m, 1H, H-1'eq), 4.12 (dd, *J* = 13.1, 2.9 Hz, 1H, H-1'ax), 3.99 (dd, *J* = 43.6, 11.8 Hz, 2H, CH₂-4'Bn), 3.86–3.84 (m, 1H, H-5'), 3.80 (dd, *J* = 9.8, 6.8 Hz, 1H, H-6'), 3.64–3.61 (m, 2H, H-6'' and H-4'), 2.67–2.63 (m, 1H, H-3'eq), 2.04–2.00 (m, 1H, H-3'ax); ¹³C NMR (150 MHz, CDCl₃) δ 164.6 (CO-Bz), 152.2 (C-6), 151.6 (C-2), 149.7 (C-4), 144.3 (C-8), 137.8, 137.1 (2 × ipso C-Bn), 128.8, 128.6, 128.4, 128.2, 127.8, 127.6, 127.3 (Ar C-Bn), 121.9 (C-5), 79.0 (C-5'), 73.6 (CH₂-6'Bn), 71.1 (CH₂-4'Bn), 70.3 (C-4'), 70.0 (C-6'), 69.6 (C-1'), 47.3 (C-2'), 29.4 (C-3'); HRMS (ESI-TOF) *m/z*: [M + H]⁺ calcd for C₃₂H₃₁N₅O₄, 550.2449; found, 550.2466.

1',5'-Anhydro-2',3'-dideoxy-2'-(adenin-9-yl)-D-allo-hexitol (32). To a stirred solution of **31** (25.5 g, 46.39 mmol) in anhydrous CH₂Cl₂ (300 mL) was added 1 M BCl₃ in CH₂Cl₂ (139.2 mL, 139.18 mmol) dropwise at –78 °C under argon. The reaction mixture then was slowly warmed to room temperature and left stirring for 3 h at the same temperature. Upon completion, the reaction mixture again cooled to –40 °C and was quenched with methanol (200 mL). Volatiles were concentrated in vacuo to provide a crude mixture of **32**

and **33**. The resulting mixture of **32** and **33** was stirred with 7 N NH₃ in MeOH (200 mL) at room temperature for 24 h. The reaction mixture was concentrated under reduced pressure, and the resulting crude residue was purified by column chromatography on silica gel (*R*_f = 0.25, 15% MeOH in CH₂Cl₂; gradient CH₂Cl₂/MeOH, 24:1, v/v; 9:1, v/v; 6:1, v/v) to give **32** (10.58 g, 86%) as a white solid. Mp 228 °C. ¹H NMR (600 MHz, DMSO-*d*₆) δ 8.56 (s, 1H, H-8), 8.23 (s, 1H, H-2), 7.66 (br s, 2H, NH₂-6), 4.62–4.60 (m, 1H, H-2'), 4.20–4.17 (m, 1H, H-1'eq), 3.94 (dd, *J* = 12.7, 3.2 Hz, 1H, H-1'ax), 3.80–3.79 (m, 1H, H-4'), 3.63 (dd, *J* = 11.4, 7.0 Hz, 1H, H-6'), 3.57 (dd, *J* = 11.3, 5.1 Hz, 1H, H-6''), 3.54–3.51 (m, 1H, H-5'), 2.24–2.20 (m, 1H, H-3'eq), 2.17–2.13 (m, 1H, H-3'ax); ¹³C NMR (150 MHz, DMSO-*d*₆) δ 154.4 (C-6), 150.2 (C-2), 149.2 (C-4), 141.9 (C-8), 117.8 (C-5), 80.3 (C-5'), 68.5 (C-1'), 62.5 (C-4'), 60.7 (C-6'), 47.3 (C-2'), 33.8 (C-3'); HRMS (ESI-TOF) *m/z*: [M + H]⁺ calcd for C₁₁H₁₅N₅O₃, 266.1247; found, 266.1260.

1',5'-Anhydro-2',3'-dideoxy-2'-(N⁶-benzoyladenin-9-yl)-D-allo-hexitol (33). To a stirred solution of **32** (10.58 g, 39.88 mmol) in anhydrous pyridine (190 mL) was added TMSCl (30.37 mL, 239.3 mmol) dropwise at 0 °C under argon. The reaction mixture then was slowly warmed to room temperature and left stirring for 5 h at the same temperature. The reaction mixture was again cooled to 0 °C, and benzoyl chloride (11.58 mL, 99.7 mmol) was added to it and stirred at room temperature for 16 h. The reaction mixture was cooled to 0 °C and quenched with 5% NaHCO₃ solution and diluted with water (500 mL), and the aq layer was extracted with CH₂Cl₂ (3 × 500 mL). The combined organic layer was dried over Na₂SO₄, filtered, and concentrated in vacuo. The resulting crude was dissolved in THF (200 mL) in a plastic vessel, and Et₃N·3HF (19.5 mL, 119.64 mmol) was added and stirred at room temperature for 16 h. The reaction mixture was concentrated in reduced pressure, and crude was partitioned between 5% NaHCO₃ solution (400 mL) and CH₂Cl₂ (400 mL). The aq layer was extracted with CH₂Cl₂ (2 × 400 mL). The combined organic layer was dried over Na₂SO₄, filtered, and concentrated in vacuo. The resulting crude was stirred with 2 N NH₃ in EtOH (150 mL) at 0 °C for 0.75 h. The reaction mixture was concentrated in reduced pressure (bath temp 12 °C), and crude residue was purified by column chromatography on silica gel (*R*_f = 0.30, 10% MeOH in CH₂Cl₂; gradient CH₂Cl₂/MeOH, 49:1, v/v; 24:1, v/v; 23:2, v/v) to give **33** (9.87 g, 67% in four steps) as a white solid. Mp 196 °C. ¹H NMR (600 MHz, DMSO-*d*₆) δ 11.09 (NH-6), 8.76 (s, 1H, H-8), 8.72 (s, 1H, H-2), 8.05 (d, *J* = 7.8 Hz, 2H, *o*H-Bz), 7.64 (t, *J* = 7.4 Hz, 1H, *p*H-Bz), 7.55 (t, *J* = 8.0 Hz, 2H, *m*H-Bz), 4.76–4.74 (m, 1H, H-2'), 4.70 (d, *J* = 3.5 Hz, 1H, OH-4'), 4.65–4.63 (m, 1H, OH-6'), 4.31–4.28 (m, 1H, H-1'eq), 4.00 (dd, *J* = 12.8, 3.1 Hz, 1H, H-1'ax), 3.81–3.79 (m, 1H, H-4'), 3.66–3.62 (m, 1H, H-6'), 3.59–3.54 (m, 2H, H-6'' and H-5'), 2.27–2.24 (m, 1H, H-3'eq), 2.23–2.19 (m, 1H, H-3'ax); ¹³C NMR (150 MHz, DMSO-*d*₆) δ 165.6 (CO-Bz), 152.4 (C-6), 151.1 (C-2), 149.8 (C-4), 144.9 (C-8), 133.6 (ipso C-Bz), 132.4 (*p*C-Bz), 128.5 (*m*C-Bz), 128.5 (*o*C-Bz), 125.1 (C-5), 80.4 (C-5'), 68.6 (C-1'), 62.6 (C-4'), 61.0 (C-6'), 47.4 (C-2'), 33.7 (C-3'); HRMS (ESI-TOF) *m/z*: [M + H]⁺ calcd for C₁₈H₁₉N₅O₄, 370.1510; found, 370.1506.

1',5'-Anhydro-6'-O-tert-butylphenylsilyl-2',3'-dideoxy-2'-(N⁶-benzoyladenin-9-yl)-D-allo-hexitol (34). A synthetic protocol similar to that used for the synthesis of **10** was employed for the synthesis of **34**, starting from **33** (9.87 g, 26.72 mmol) and TBDPSCl (10.4 mL, 40.08 mmol) in anhydrous pyridine (150 mL) to obtain **34** (13 g, 80%) as a white foam (*R*_f = 0.64, 10% MeOH in CH₂Cl₂; column chromatography gradient CH₂Cl₂/MeOH, 100:0, v/v; 99:1, v/v; 39:1, v/v). Mp 123 °C. ¹H NMR (500 MHz, CDCl₃) δ 9.21 (br s, 1H, NH-6), 8.74 (s, 1H, H-8), 8.70 (s, 1H, H-2), 8.00–7.98 (m, 2H, *o*H-Bz), 7.71–7.66 (m, 4H, Ar-H TBDPS), 7.55–7.52 (m, 1H, *p*H-Bz), 7.46 (t, *J* = 7.7 Hz, 2H, *m*H-Bz), 7.44–7.37 (m, 6H, Ar-H TBDPS), 4.77–4.75 (m, 1H, H-2'), 4.43 (dd, *J* = 12.9, 1.9 Hz, 1H, H-1'eq), 4.13 (br s, 1H, H-4'), 4.05 (dd, *J* = 13.0, 2.9 Hz, 1H, H-1'ax), 3.97 (d, *J* = 5.0 Hz, 2H, H-6' and H-6''), 3.62 (br s, 1H, OH-4'), 3.59–3.57 (m, 1H, H-5'), 2.50–2.45 (m, 1H, H-3'eq), 2.21–2.16 (m, 1H, H-3'ax), 1.06 (s, 9H, 3 × CH₃-*t*Bu); ¹³C NMR (125 MHz, CDCl₃) δ 164.9 (CO-Bz), 152.1 (C-6), 151.8 (C-2), 149.1 (C-4),

144.4 (C-8), 135.7, 135.6, 134.0, 132.8, 132.6, 130.1, 128.8, 128.0 (Ar C-TBDPS and Bz), 122.2 (C-5), 79.1 (C-5'), 70.3 (C-1'), 65.0 (C-6'), 64.8 (C-4'), 47.2 (C-2'), 33.9 (C-3'), 26.9 (3 × CH₃-^tBu), 19.2 (1C ^tBu); HRMS (ESI-TOF) *m/z*: [M + H]⁺ calcd for C₃₄H₃₇N₃O₃Si, 608.2687; found, 608.2691.

1',5'-Anhydro-6'-O-tert-butylidiphenylsilyl-4'-O-mesyl-2',3'-dideoxy-2'-(N⁶-benzoyladenine-9-yl)-D-allo-hexitol (35). A synthetic protocol similar to that used for the synthesis of **11** was employed for the synthesis of **35**, starting from **34** (13 g, 21.39 mmol) and MsCl (4.97 mL, 64.17 mmol) in anhydrous pyridine (230 mL) to obtain **35** (12.5 g, 85%) as a white foam (*R_f* = 0.45, 6% MeOH in CH₂Cl₂; column chromatography gradient CH₂Cl₂/MeOH, 100:0, v/v; 99:1, v/v; 66:1, v/v). Mp 148 °C. ¹H NMR (300 MHz, CDCl₃) δ 9.25 (br s, 1H, NH-6), 8.74 (s, 1H, H-2), 8.50 (s, 1H, H-8), 8.02–7.37 (m, 15H, Ar-H TBDPS and Bz), 5.03–5.01 (m, 1H, H-2'), 4.82 (br s, 1H, H-4'), 4.48 (d, *J* = 13.0 Hz, 1H, H-1'_{eq}), 4.10–4.05 (m, 1H, H-1'_{ax}), 3.88–3.73 (m, 3H, H-5', H-6', and H-6''), 2.97–2.90 (m, 1H, H-3'_{eq}), 2.35–2.25 (m, 1H, H-3'_{ax}), 2.50 (s, 3H, CH₃-Ms), 1.07 (s, 9H, 3 × CH₃-^tBu); ¹³C NMR (75 MHz, CDCl₃) δ 164.8 (CO-Bz), 152.5 (C-6), 152.2 (C-2), 149.4 (C-4), 143.1 (C-8), 135.7, 135.6, 132.7, 130.1, 128.9, 128.0, 127.9 (Ar C-TBDPS and Bz), 122.7 (C-5), 79.1 (C-5'), 71.3 (C-1'), 69.4 (C-6'), 62.4 (C-4'), 46.9 (C-2'), 38.4 (CH₃-Ms), 32.6 (C-3'), 26.9 (3 × CH₃-^tBu), 19.3 (1C ^tBu); HRMS (ESI-TOF) *m/z*: [M + H]⁺ calcd for C₃₅H₃₉N₃O₆SSi, 686.2463; found, 686.2455.

1',5'-Anhydro-6'-O-tert-butylidiphenylsilyl-4'-amino-2',3'-dideoxy-2'-(N⁶-benzoyladenine-9-yl)-D-alto-hexitol (38). To a stirred solution of compound **35** (12.5 g, 18.22 mmol) in dry DMF (200 mL) was added sodium azide (5.34 g, 82.01 mmol), and the resulting reaction mixture was heated at 65 °C for 72 h. The progress of the reaction was monitored by TLC, and, after completion, the reaction mixture was cooled and volatiles were removed under reduced pressure. The crude residue was suspended in 10% MeOH in CH₂Cl₂, solid residue was filtered off, washed, and filtrate was concentrated in vacuo to give a mixture of compounds (**36**, **37**, and desilylated analogues of **36** and **37**). Without purification, the resulting crude was treated with TBDPSCI (4.74 mL, 18.22 mmol) in anhydrous pyridine (200 mL) at 45 °C for 16 h. The reaction mixture was then cooled to 0 °C and neutralized with 5% NaHCO₃ solution. The aq layer was extracted with CH₂Cl₂ (3 × 500 mL). The combined organic layer was dried over Na₂SO₄, filtered, and concentrated under reduced pressure, and the resulting crude residue was purified by column chromatography on silica gel (*R_f* = 0.56, 6% MeOH in CH₂Cl₂; gradient CH₂Cl₂/MeOH, 100:0, v/v; 99:1, v/v; 66:1, v/v) to give a nonseparable mixture of **36** and **37** (10.12 g) as a white foam. HRMS (ESI-TOF) *m/z*: [M + H]⁺ calcd for C₃₄H₃₆N₈O₃Si, 633.2752; found, 633.2756.

To a stirred solution of a **36** and **37** mixture (10.12 g, 1 equiv) in MeOH (350 mL, degassed with argon) were added three drops of glacial AcOH followed by 10% Pd/C (Degussa type, 1.52 g, 0.15 equiv w/w), and evacuation was then carried out with hydrogen atmosphere replacements (3×). The reaction mixture was stirred at room temperature for 16 h under an atmospheric pressure of hydrogen. After completion of the reaction, the catalyst was removed by filtration through a Celite pad, and the pad was thoroughly washed with MeOH. The combined filtrate was concentrated under reduced pressure, and the crude residue was purified by column chromatography on silica gel (*R_f* = 0.27, 10% MeOH in CH₂Cl₂; gradient CH₂Cl₂/MeOH, 49:1, v/v; 24:1, v/v; 19:1, v/v) to afford **38** (2.21 g, 20% over two steps) as a white foam. Mp 144 °C. ¹H NMR (500 MHz, CDCl₃) δ 9.36 (br s, 1H, NH-6), 8.81 (s, 1H, H-2), 8.65 (s, 1H, H-8), 8.03 (d, *J* = 7.6 Hz, 2H, oH-Bz), 7.73–7.69 (m, 4H, Ar H-TBDPS), 7.59 (t, *J* = 7.4 Hz, 1H, pH-Bz), 7.51 (t, *J* = 7.5 Hz, 2H, mH-Bz), 7.45–7.38 (m, 6H, Ar-H TBDPS), 4.96 (br s, 1H, H-2'), 4.36 (d, *J* = 12.8 Hz, 1H, H-1'_{eq}), 3.99–3.91 (m, 3H, H-1'_{ax}, H-6', and H-6''), 3.25–3.22 (m, 1H, H-5'), 3.13–3.08 (m, 1H, H-4'), 2.43–2.38 (m, 1H, H-3'_{eq}), 1.89–1.83 (m, 1H, H-3'_{ax}), 1.77 (br s, 2H, NH₂-4'), 1.10 (s, 9H, 3 × CH₃-^tBu); ¹³C NMR (125 MHz, CDCl₃) δ 164.7 (CO-Bz), 152.4 (C-6), 151.7 (C-2), 149.5 (C-4), 142.8 (C-8), 135.6, 135.5, 133.8, 133.0, 132.9, 132.6, 129.9, 129.8,

128.7, 128.7, 127.9, 127.8, 127.7 (Ar C-TBDPS and Bz), 122.3 (C-5), 84.5 (C-5'), 69.2 (C-1'), 63.9 (C-6'), 50.4 (C-2'), 43.4 (C-4'), 37.0 (C-3'), 26.9 (3 × CH₃-^tBu), 19.2 (1C ^tBu); HRMS (ESI-TOF) *m/z*: [M + H]⁺ calcd for C₃₄H₃₈N₆O₃Si, 607.2847; found, 607.2874.

1',5'-Anhydro-6'-O-tert-butylidiphenylsilyl-4'-amino(trityl)-2',3'-dideoxy-2'-(N⁶-benzoyladenine-9-yl)-D-alto-hexitol (39). To a stirred solution of compound **38** (2.21 g, 3.64 mmol) in dry CH₂Cl₂ (60 mL) were added triethylamine (5.06 mL, 36.4 mmol) followed by trityl chloride (3.05 g, 10.94 mmol), and the resulting reaction mixture was stirred at 40 °C for 16 h. The progress of the reaction was monitored by TLC, and, after completion, the reaction mixture was cooled and quenched with 5% NaHCO₃ solution. The aq layer was extracted with CH₂Cl₂ (3 × 100 mL). The combined organic layer was dried over Na₂SO₄, filtered and concentrated under reduced pressure, and the resulting crude residue was purified by column chromatography on silica gel (*R_f* = 0.72, EtOAc; gradient hexane/EtOAc, 4:1, v/v; 3:2, v/v; 2:3, v/v) to give **39** (2.48 g, 80%) as a white foam. Mp 152 °C. ¹H NMR (500 MHz, CDCl₃) δ 9.24 (br s, 1H, NH-6), 8.74 (s, 1H, H-2), 8.08–8.06 (m, 3H, H-8 and Ar H-anisyl), 7.67–7.03 (m, 28H, Ar H-TBDPS, Bz and Trt), 4.67–4.65 (m, 1H, H-2'), 4.12 (dd, *J* = 14.3, 7.2 Hz, 1H, H-1'_{eq}), 3.91 (dd, *J* = 12.5, 3.2 Hz, 1H, H-1'_{ax}), 3.88–3.83 (m, 2H, H-6' and H-6''), 3.44–3.41 (m, 1H, H-5'), 2.61–2.58 (m, 1H, H-4'), 2.29 (br s, 1H, NH-4'), 1.80–1.70 (m, 2H, H-3'_{eq} and H-3'_{ax}), 1.02 (s, 9H, 3 × CH₃-^tBu); ¹³C NMR (125 MHz, CDCl₃) δ 164.6 (CO-Bz), 152.2 (C-6), 151.3 (C-2), 149.2 (C-4), 145.9 (*ipso* C-Trt), 142.2 (C-8), 135.6, 135.5, 133.8, 133.0, 132.9, 132.7, 129.8, 129.7, 128.8, 128.5, 127.8, 127.7, 127.6, 126.3 (Ar C-TBDPS, Trt and Bz), 122.3 (C-5), 81.8 (C-5'), 70.4 (1C-Trt), 67.9 (C-1'), 64.4 (C-6'), 49.8 (C-2'), 48.5 (C-4'), 34.6 (C-3'), 26.8 (3 × CH₃-^tBu), 19.1 (1C ^tBu); HRMS (ESI-TOF) *m/z*: [M + H]⁺ calcd for C₅₃H₅₂N₆O₃Si, 849.3943; found, 849.3931.

1',5'-Anhydro-4'-amino(trityl)-2',3'-dideoxy-2'-(N⁶-benzoyladenine-9-yl)-D-alto-hexitol (3). A synthetic protocol similar to that used for the synthesis of **2** was employed for the synthesis of **3**, starting from **39** (2.48 g, 2.92 mmol) and triethylamine trihydrofluoride (1.90 mL, 11.68 mmol) in anhydrous THF (60 mL) at 46 °C for 72 h to obtain **3** (1.55 g, 87%) as a white semisolid (*R_f* = 0.34, 5% MeOH in EtOAc; column chromatography gradient EtOAc/MeOH, 100:0, v/v; 99:1, v/v; 49:1, v/v). Mp 192 °C. ¹H NMR (600 MHz, DMSO-*d*₆) δ 11.16 (br s, 1H, NH-6), 8.68 (s, 1H, H-2), 8.22 (m, 1H, H-8), 8.09 (d, *J* = 7.7 Hz, 2H, oH-Bz), 7.65 (t, *J* = 7.5 Hz, 1H, pH-Bz), 7.57 (t, *J* = 7.8 Hz, 2H, mH-Bz), 7.32–7.05 (m, 15H, Ar H-Trt), 4.77 (br s, 1H, OH-6'), 4.67–4.65 (m, 1H, H-2'), 4.17 (d, *J* = 12.3 Hz, 1H, H-1'_{eq}), 3.88 (dd, *J* = 12.5, 3.1 Hz, 1H, H-1'_{ax}), 3.68–3.64 (m, 1H, H-6'), 3.58–3.55 (m, 1H, H-6''), 3.45–3.42 (m, 1H, H-5'), 3.10 (d, *J* = 5.2 Hz, 1H, NH-4'), 2.31–2.27 (m, 1H, H-4'), 1.71–1.63 (m, 2H, H-3'_{eq} and H-3'_{ax}); ¹³C NMR (150 MHz, DMSO-*d*₆) δ 165.7 (CO-Bz), 152.1 (C-6), 151.1 (C-2), 150.1 (C-4), 146.7 (*ipso* C-Trt), 143.1 (C-8), 133.6, 132.4, 128.5, 128.4, 127.5, 126.2 (Ar C-Trt and Bz), 125.4 (C-5), 80.9 (C-5'), 70.1 (1C-Trt), 67.0 (C-1'), 61.9 (C-6'), 49.7 (C-2'), 48.2 (C-4'), 33.9 (C-3'); HRMS (ESI-TOF) *m/z*: [M + H]⁺ calcd for C₃₇H₃₄N₆O₃, 611.2765; found, 611.2761.

1',5'-Anhydro-4',6'-di-O-benzyl-2'-deoxy-2'-(2-amino-6-chloropurin-9-yl)-D-allo-hexitol (40). To a stirred solution of **26** (45 g, 137.9 mmol) and adenine (28.1 g, 165.5 mmol) in dry DMSO (450 mL) was added DBU (26.8 mL, 179.3 mmol) under argon, and the reaction mixture was heated at 88 °C for 36 h. The reaction was monitored with TLC; upon completion, the reaction mixture was cooled and the residue was diluted with ice-water (4 L) and neutralized with 0.5 N HCl at 0 °C. The aqueous layer was extracted with EtOAc (3 × 800 mL). The combined organic layer was dried over Na₂SO₄, filtered and concentrated in vacuo, and the crude residue was purified by column chromatography on silica gel (*R_f* = 0.42, 8% MeOH in CH₂Cl₂; gradient CH₂Cl₂/MeOH, 99:1, v/v; 49:1, v/v; 39:1, v/v) to give **40** (41.03 g, 60%) as a white solid. Mp 153 °C. ¹H NMR (500 MHz, CDCl₃) δ 8.20 (s, 1H, H-8), 7.36–6.91 (m, 10H, 2 × Ar H-Bn), 5.15 (br s, 2H, NH₂-2), 4.58 (dd, *J* = 36.9, 11.9 Hz, 2H, CH₂-6'Bn), 4.35–4.19 (m, 7H, CH₂-4'Bn, H-2', H-1'_{eq}, H-1'_{ax}, H-5', and H-3'), 3.85 (dd, *J* = 10.2, 7.1 Hz, 1H, H-6'), 3.63

(dd, $J = 10.2, 4.6$ Hz, 1H, H-6''), 3.51 (t, $J = 3.5$ Hz, 1H, H-4' ^{13}C NMR (125 MHz, CDCl_3) δ 158.7 (C-2), 153.7 (C-4), 151.0 (C-6), 143.2 (C-8), 137.8, 137.0 (2 $\times$ *ipso* C-Bn), 128.5, 128.3, 128.0, 127.9 (Ar C-Bn), 124.7 (C-5), 76.5 (C-4'), 74.1 (CH_2 -6'Bn), 73.6 (CH_2 -4'Bn), 73.3 (C-5'), 68.8 (C-6'), 65.7 (C-3'), 64.1 (C-1'), 53.9 (C-2'); HRMS (ESI-TOF) m/z : $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{25}\text{H}_{26}\text{ClN}_5\text{O}_4$, 496.1746; found, 496.1747.

1',5'-Anhydro-4',6'-di-O-benzyl-2'-deoxy-2'-(guanin-9-yl)-D-allo-hexitol (41). To a stirred solution of **40** (41 g, 82.66 mmol) in 2 N NaOH and 1,4-dioxane (1:1, 450 mL) was added DABCO (2.72 g, 24.25 mmol), and the reaction mixture was heated at 90 °C for 16 h. The reaction was monitored with TLC; upon completion, the reaction mixture was cooled and volatiles were removed under reduced pressure. The resulting crude was diluted with ice-cold water, and the pH was adjusted to neutral with 1 N HCl at 0 °C. The solid formed was filtered off, washed with water, 3% MeOH in CH_2Cl_2 , and dried in vacuo ($R_f = 0.13$, 15% MeOH in CH_2Cl_2) to give **41** (32.8 g, 83%) as a white solid. ^1H NMR (600 MHz, $\text{DMSO}-d_6$) δ 10.89 (br s, 1H, NH-1), 8.10 (s, 1H, H-8), 7.36–7.01 (m, 10H, 2 $\times$ Ar H-Bn), 6.67 (br s, 2H, NH-2), 4.51 (dd, $J = 37.1, 12.0$ Hz, 2H, CH_2 -6'Bn), 4.32 (dd, $J = 31.5, 11.8$ Hz, 2H, CH_2 -4'Bn), 4.19–4.15 (m, 2H, H-2' and H-3'), 4.12–4.08 (m, 2H, H-1' $_{\text{eq}}$ and H-5'), 4.01 (dd, $J = 12.4, 3.2$ Hz, 1H, H-1' $_{\text{ax}}$), 3.77 (dd, $J = 10.2, 7.4$ Hz, 1H, H-6'), 3.61 (dd, $J = 10.5, 4.8$ Hz, 1H, H-6''), 3.45 (t, $J = 3.6$ Hz, 1H, H-4'); ^{13}C NMR (150 MHz, $\text{DMSO}-d_6$) δ 156.0 (C-6), 154.0 (C-2), 150.9 (C-4), 138.4, 138.1 (2 $\times$ *ipso* C-Bn), 137.1 (C-8), 128.3, 128.3, 128.1, 128.0, 127.7, 127.6, 127.5, 127.4 (Ar C-Bn), 113.7 (C-5), 77.0 (C-4'), 73.7 (C-5'), 72.4 (CH_2 -6'Bn), 72.2 (CH_2 -4'Bn), 68.1 (C-6'), 64.8 (C-3'), 63.8 (C-1'), 53.3 (C-2'); HRMS (ESI-TOF) m/z : $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{25}\text{H}_{27}\text{N}_5\text{O}_5$, 478.2085; found, 478.2077.

1',5'-Anhydro-4',6'-di-O-benzyl-2'-deoxy-2'-(N²-iso-butrylguanin-9-yl)-D-allo-hexitol (42). A synthetic protocol similar to that used for the synthesis of **33** was employed for the synthesis of **42**, starting from **41** (32.8 g, 68.69 mmol), TMSCl (34.9 mL, 274.76 mmol) in anhydrous pyridine (500 mL); isobutyric anhydride (47.8 mL, 288.5 mmol) in a second step; triethylamine trihydrofluoride (16.8 mL, 103.03 mmol) in anhydrous THF (400 mL) in a third step; and N NH_3 in EtOH (300 mL) in a fourth step to obtain **42** (31.6 g, 84% over four steps) as a white semisolid ($R_f = 0.30$, 10% MeOH in CH_2Cl_2 ; column chromatography gradient $\text{CH}_2\text{Cl}_2/\text{MeOH}$, 100:0, v/v; 99:1, v/v; 39:1, v/v). Mp 201 °C. ^1H NMR (500 MHz, CDCl_3) δ 12.48 (br s, 1H, NH-1), 11.00 (br s, 1H, NH-2), 8.35 (s, 1H, H-8), 7.31–6.86 (m, 10H, 2 $\times$ Ar H-Bn), 6.07 (br s, 1H, OH-3'), 4.52 (dd, $J = 53.6, 12.0$ Hz, 2H, CH_2 -6'Bn), 4.44–4.40 (m, 3H, H-1' $_{\text{eq}}$, H-2', and H-3'), 4.26–4.23 (m, 2H, and H-5'), 4.18 (d, $J = 11.8$ Hz, 1H, H-1' $_{\text{ax}}$), 4.14 (s, 2H, CH_2 -4'Bn), 3.78 (dd, $J = 10.0, 7.7$ Hz, 1H, H-6'), 3.51–3.48 (m, 2H, H-6' and H-4'), 2.92 (p, $J = 6.8$ Hz, 1H, CH- ^iPr), 1.27 (t, $J = 6.7$ Hz, 6H, 2 $\times$ CH_3 - ^iPr); ^{13}C NMR (125 MHz, CDCl_3) δ 180.4 (CO- ^iBu), 156.1 (C-6), 149.5 (C-2), 148.0 (C-4), 140.7 (C-8), 137.9, 137.5 (2 $\times$ *ipso* C-Bn), 128.4, 128.1, 127.7, 127.6, 127.5, 127.4 (Ar C-Bn), 118.9 (C-5), 76.0 (C-4'), 74.4 (C-5'), 73.3 (CH_2 -6'Bn), 72.4 (CH_2 -4'Bn), 70.0 (C-6'), 64.9 (C-3'), 64.2 (C-1'), 53.5 (C-2'), 36.1 (CH- ^iPr), 19.1, 19.0 (2 $\times$ CH_3 - ^iPr); HRMS (ESI-TOF) m/z : $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{29}\text{H}_{33}\text{N}_5\text{O}_6$, 548.2503; found, 548.2513.

1',5'-Anhydro-4',6'-di-O-benzyl-3',2'-dideoxy-2'-(N²-iso-butrylguanin-9-yl)-D-allo-hexitol (44). A slightly modified synthetic protocol from that used for the synthesis of **7** was employed for the synthesis of **43**, starting from **42** (31.6 g, 57.7 mmol), aq NaOH (50% w/v; 22.6 mL), CS_2 (33.9 mL), and 3-bromo propionitrile (33.9 mL) in DMSO (700 mL) in a first step to obtain 1',5'-anhydro-4',6'-di-O-benzyl-3'-(2-cyanoethyl)carbonodithioate-2'-deoxy-2'-(N²-iso-butrylguanin-9-yl)-D-allo-hexitol xanthate **43** (31.22 g, 80%) as an off-white semisolid ($R_f = 0.51$, 10% MeOH in CH_2Cl_2 ; column chromatography gradient $\text{CH}_2\text{Cl}_2/\text{MeOH}$, 100:0, v/v; 99:1, v/v; 49:1, v/v). ^1H NMR (600 MHz, CDCl_3) δ 11.97 (br s, 1H, NH-1), 8.72 (br s, 1H, NH-2), 8.10 (s, 1H, H-8), 7.37–7.00 (m, 10H, 2 $\times$ Ar H-Bn), 6.17 (br s, 1H, H-3'), 4.57–4.45 (m, 3H, CH_2 -6'Bn and H-2'), 4.43–4.40 (m, 1H, H-1' $_{\text{eq}}$), 4.34 (dd, $J = 17.2, 12.0$ Hz, 2H, CH_2 -4'Bn, and H-3'), 4.21 (dd, $J = 13.0, 3.6$ Hz, 1H, H-1' $_{\text{ax}}$),

3.97–3.95 (m, 1H, H-5'), 3.78 (dd, $J = 10.1, 6.4$ Hz, 1H, H-6' 3.67 (t, $J = 2.8$ Hz, 1H, H-4'), 3.60–3.33 (m, 3H, $\text{SCH}_2\text{CH}_2\text{CN}$ and H-6''), 2.91–2.77 (m, 2H, $\text{SCH}_2\text{CH}_2\text{CN}$), 2.63 (p, $J = 6.8$ Hz, 1H, CH- ^iPr), 1.27–1.24 (m, 6H, 2 $\times$ CH_3 - ^iPr); ^{13}C NMR (150 MHz, CDCl_3) δ 211.7 (OCS₂), 178.5 (CO- ^iBu), 155.5 (C-6), 148.1 (C-2), 147.4 (C-4), 139.1 (C-8), 137.5, 136.7 (2 $\times$ *ipso* C-Bn), 128.5, 128.3, 128.1, 128.0, 128.0, 127.9 (Ar C-Bn), 120.2 (CN), 117.3 (C-5), 75.7 (C-3'), 75.3 (C-5'), 73.8 (CH_2 -6'Bn), 72.7 (CH_2 -4'Bn), 71.7 (C-4'), 69.6 (C-6'), 65.9 (C-1'), 50.1 (C-2'), 40.9 (CH- ^iPr), 31.4 ($\text{SCH}_2\text{CH}_2\text{CN}$), 19.0, 18.9 (2 $\times$ CH_3 - ^iPr), 17.8 ($\text{SCH}_2\text{CH}_2\text{CN}$); HRMS (ESI-TOF) m/z : $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{33}\text{H}_{36}\text{N}_6\text{O}_6\text{S}_2$, 677.2210; found, 677.2217.

A Barton–McCombie condition similar to that used for the synthesis of **8** was employed for the synthesis of **44**, starting from **43** (31.22 g, 46.17 mmol), $n\text{Bu}_3\text{SnH}$ (33.1 mL, 122.8 mmol), and AIBN (1.52 g, 9.23 mmol) in anhydrous toluene (620 mL) to obtain **44** (20.13 g, 82%) as a white solid ($R_f = 0.39$, 10% MeOH in CH_2Cl_2 ; column chromatography gradient $\text{CH}_2\text{Cl}_2/\text{MeOH}$, 99:1, v/v; 49:1, v/v; 39:1, v/v). Mp 78 °C. ^1H NMR (600 MHz, CDCl_3) δ 12.14 (br s, 1H, NH-1), 9.87 (br s, 1H, NH-2), 8.35 (s, 1H, H-8), 7.32–6.94 (m, 10H, 2 $\times$ Ar H-Bn), 4.50 (dd, $J = 61.8, 11.9$ Hz, 2H, CH_2 -6'Bn), 4.42–4.40 (m, 1H, H-2'), 4.25 (d, $J = 12.9$ Hz, 1H, H-1' $_{\text{eq}}$), 4.06 (dd, $J = 39.8, 11.9$ Hz, 2H, CH_2 -4'Bn), 3.94 (dd, $J = 39.8, 11.9$ Hz, 1H, H-1' $_{\text{ax}}$), 3.76–3.71 (m, 2H, H-6' and H-5'), 3.57–3.54 (m, 2H, H-6' and H-4'), 2.89–2.83 (m, 1H, CH- ^iPr), 2.44–2.40 (m, 1H, H-3' $_{\text{eq}}$), 1.90–1.87 (m, 1H, H-3' $_{\text{ax}}$), 1.11, 1.10 (2d, $J = 6.8$ Hz, 6H, 2 $\times$ CH_3 - ^iPr); ^{13}C NMR (150 MHz, CDCl_3) δ 179.3 (CO- ^iBu), 155.9 (C-6), 148.4 (C-2), 147.4 (C-4), 140.1 (C-8), 137.8, 137.3 (2 $\times$ *ipso* C-Bn), 128.4, 128.2, 127.7, 127.7, 127.6 (Ar C-Bn), 119.9 (C-5), 78.7 (C-5'), 73.5 (CH_2 -6'Bn), 71.1 (CH_2 -4'Bn), 70.4 (C-4'), 69.9 (C-6'), 69.5 (C-1'), 47.0 (C-2'), 36.1 (CH- ^iPr), 29.5 (C-3'), 19.1, 19.0 (2 $\times$ CH_3 - ^iPr); HRMS (ESI-TOF) m/z : $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{29}\text{H}_{33}\text{N}_5\text{O}_5$, 532.2554; found, 532.2559.

1',5'-Anhydro-3',2'-dideoxy-2'-(N²-iso-butrylguanin-9-yl)-D-allo-hexitol (45). To a stirred solution of **44** (20.13 g, 37.87 mmol) in anhydrous CH_2Cl_2 (400 mL) was added 1 M BCl_3 in CH_2Cl_2 (151.5 mL, 151.5 mmol) dropwise at -78 °C under argon. The reaction mixture then was slowly warmed to room temperature and left stirring for 3 h at same temperature. Upon completion, the reaction mixture was again cooled to -40 °C and quenched with methanol (300 mL). Volatiles were concentrated in reduced pressure, and the resulting crude residue was purified by column chromatography on silica gel ($R_f = 0.17$, 15% MeOH in CH_2Cl_2 ; gradient $\text{CH}_2\text{Cl}_2/\text{MeOH}$, 24:1, v/v; 9:1, v/v; 6:1, v/v) to give **45** (11.84 g, 89%) as a white solid. Mp 215 °C. ^1H NMR (600 MHz, $\text{DMSO}-d_6$) δ 12.01 (br s, 1H, NH-1), 11.70 (br s, 1H, NH-2), 8.31 (s, 1H, H-8), 4.83 (d, $J = 3.4$ Hz, 1H, OH-4'), 4.72 (t, $J = 5.9$ Hz, 1H, OH-6'), 4.43–4.41 (m, 1H, H-2'), 4.13 (d, $J = 12.5$ Hz, 1H, H-1' $_{\text{eq}}$), 3.91 (dd, $J = 12.6, 3.0$ Hz, 1H, H-1' $_{\text{ax}}$), 3.79–3.77 (m, 1H, H-4'), 3.63–3.51 (m, 2H, H-6' and H-6''), 3.50–3.48 (m, 1H, H-5'), 2.79 (p, $J = 6.8$ Hz, 1H, CH- ^iPr), 2.20–2.17 (m, 1H, H-3' $_{\text{eq}}$), 2.14–2.10 (m, 1H, H-3' $_{\text{ax}}$), 1.25 (d, $J = 6.9$ Hz, 6H, 2 $\times$ CH_3 - ^iPr); ^{13}C NMR (150 MHz, $\text{DMSO}-d_6$) δ 180.1 (CO- ^iBu), 155.1 (C-6), 148.5 (C-2), 147.6 (C-4), 140.1 (C-8), 119.3 (C-5), 80.2 (C-5'), 68.6 (C-1'), 62.3 (C-4'), 60.5 (C-6'), 47.4 (C-2'), 34.8 (CH- ^iPr), 33.9 (C-3'), 19.0 (2 $\times$ CH_3 - ^iPr); HRMS (ESI-TOF) m/z : $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{15}\text{H}_{21}\text{N}_5\text{O}_5$, 352.1615; found, 352.1612.

1',5'-Anhydro-6'-O-tert-butylidiphenylsilyl-3',2'-dideoxy-2'-(N²-iso-butrylguanin-9-yl)-D-allo-hexitol (46). A synthetic protocol similar to that used for the synthesis of **10** was employed for the synthesis of **46**, starting from **45** (11.84 g, 33.70 mmol) and TBDPSCI (17.5 mL, 67.39 mmol) in anhydrous pyridine (230 mL) to obtain **46** (14.91 g, 75%) as a white foam ($R_f = 0.46$, 10% MeOH in CH_2Cl_2 ; column chromatography gradient $\text{CH}_2\text{Cl}_2/\text{MeOH}$, 99:1, v/v; 49:1, v/v; 97:3, v/v). Mp 166 °C. ^1H NMR (500 MHz, CD_3OD) δ 8.40 (s, 1H, H-8), 7.73–7.38 (m, 10H, Ar H-TBDPS), 4.54–4.52 (m, 1H, H-2'), 4.29 (d, $J = 13.0$ Hz, 1H, H-1' $_{\text{eq}}$), 4.00 (dd, $J = 13.0, 3.0$ Hz, 1H, H-1' $_{\text{ax}}$), 3.91–3.90 (m, 3H, H-4', H-6', and H-6''), 3.71–3.68 (m, 1H, H-5'), 2.69 (h, $J = 6.9$ Hz, 1H, CH- ^iPr), 2.31–2.27 (m, 1H, H-3' $_{\text{eq}}$), 2.22–2.18 (m, 1H, H-3' $_{\text{ax}}$), 1.21 (d, $J = 6.9$ Hz, 6H, 2 $\times$

CH_3 - ^iPr), 1.04 (s, 9H, $3 \times \text{CH}_3$ - ^iBu); ^{13}C NMR (125 MHz, CD_3OD) δ 181.6 (CO- ^iBu), 157.5 (C-6), 150.5 (C-2), 149.2 (C-4), 142.2 (C-8), 136.8, 136.7 (Ar C-TBDPS), 134.7, 134.6 ($2 \times ipso$ C-Bn), 130.9, 128.8, 128.7 (Ar C-TBDPS), 119.9 (C-5), 81.7 (C-5'), 70.6 (C-1'), 65.6 (C-6'), 64.6 (C-4'), 49.1 (C-2'), 36.9 (CH- ^iPr), 35.2 (C-3'), 27.3 ($3 \times \text{CH}_3$ - ^iBu), 20.0 (1C- ^iBu), 19.4, 19.3 ($2 \times \text{CH}_3$ - ^iPr); HRMS (ESI-TOF) m/z : $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{31}\text{H}_{39}\text{N}_3\text{O}_5\text{Si}$, 590.2793; found, 590.2802.

1',5'-Anhydro-6'-O-tert-butylidiphenylsilyl-4'-O-mesyl-3',2'-dideoxy-2'-(N²-iso-butrylguanin-9-yl)-D-allo-hexitol (47). A synthetic protocol similar to that used for the synthesis of 11 was employed for the synthesis of 47, starting from 46 (14.91 g, 25.28 mmol) and MsCl (5.87 mL, 75.84 mmol) in anhydrous pyridine (250 mL) to obtain 35 (14.01 g, 83%) as a white foam (R_f = 0.60, 10% MeOH in CH_2Cl_2 ; column chromatography gradient $\text{CH}_2\text{Cl}_2/\text{MeOH}$, 100:0, v/v; 99:1, v/v; 97:3, v/v). Mp 194 °C. ^1H NMR (500 MHz, CDCl_3) δ 12.02 (br s, 1H, NH-1), 8.78 (br s, 1H, NH-2), 8.07 (s, 1H, H-8), 7.68–7.38 (m, 10H, Ar H-TBDPS), 4.98 (t, J = 3.0 Hz, 1H, H-4'), 4.45–4.40 (m, 1H, H-2' and H-1' $_{\text{eq}}$), 3.99 (dd, J = 13.1, 2.9 Hz, 1H, H-1' $_{\text{ax}}$), 3.81–3.75 (m, 2H, H-6' and H-6''), 3.74–3.70 (m, 1H, H-5'), 2.78–2.73 (m, 1H, H-3' $_{\text{eq}}$), 2.69 (h, J = 6.9 Hz, 1H, CH- ^iPr), 2.53 (s, 3H, CH_3 -Ms), 2.16–2.11 (m, 1H, H-3' $_{\text{ax}}$), 1.27, 1.26 (2d, J = 6.9 Hz, 6H, $2 \times \text{CH}_3$ - ^iPr), 1.06 (s, 9H, $3 \times \text{CH}_3$ - ^iBu); ^{13}C NMR (150 MHz, CDCl_3) δ 178.4 (CO- ^iBu), 155.7 (C-6), 148.3 (C-2), 147.2 (C-4), 138.8 (C-8), 135.6, 135.5 (Ar C-TBDPS), 133.0, 132.8 ($2 \times ipso$ C-Bn), 130.0, 129.9, 127.8, 127.7 (Ar C-TBDPS), 120.3 (C-5), 78.8 (C-5'), 70.6 (C-4'), 69.1 (C-1'), 62.1 (C-6'), 46.6 (C-2'), 38.3 (CH_3 -Ms), 36.5 (CH- ^iPr), 32.4 (C-3'), 26.8 ($3 \times \text{CH}_3$ - ^iBu), 19.1 (1C- ^iBu), 19.0, 18.9 ($2 \times \text{CH}_3$ - ^iPr); HRMS (ESI-TOF) m/z : $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{32}\text{H}_{41}\text{N}_5\text{O}_7\text{SSi}$, 668.2568; found, 668.2569.

1',5'-Anhydro-6'-O-tert-butylidiphenylsilyl-4'-amino-3',2'-dideoxy-2'-(N²-iso-butrylguanin-9-yl)-D-alto-hexitol (50). A synthetic protocol similar to that used for the synthesis of 36 and 37 was employed for the synthesis of 48 and 49, starting from 47 (14.01 g, 20.98 mmol) and sodium azide (6.82 g, 104.89 mmol) in anhydrous DMF (220 mL) in a first step; and TBDPSCI (5.45 mL, 20.98 mmol) in anhydrous pyridine (220 mL) in a second step to obtain a nonseparable mixture of 36 and 37 (10.9 g) as a white foam (R_f = 0.65, 10% MeOH in CH_2Cl_2 ; column chromatography gradient $\text{CH}_2\text{Cl}_2/\text{MeOH}$, 100:0, v/v; 99:1, v/v; 39:1, v/v). HRMS (ESI-TOF) m/z : $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{31}\text{H}_{38}\text{N}_8\text{O}_4\text{Si}$, 615.2858; found, 615.2833.

A synthetic protocol similar to that used for the synthesis of 38 was employed for the synthesis of 50, starting from a mixture of 36 and 37 (10.9 g), 5 drops of AcOH, and 10% Pd/C Degussa type (2.18 g, 0.2 equiv w/w) in MeOH (980 mL) to obtain 50 (3.70 g, 30% over two steps) as a white semisolid (R_f = 0.42, 10% MeOH in CH_2Cl_2 ; column chromatography gradient $\text{CH}_2\text{Cl}_2/\text{MeOH}$, 99:1, v/v; 49:1, v/v; 24:1, v/v). ^1H NMR (500 MHz, CDCl_3) δ 8.20 (s, 1H, H-8), 7.70–7.37 (m, 10H, Ar H-TBDPS), 4.56 (br s, 1H, H-2'), 4.28 (d, J = 12.9 Hz, 1H, H-1' $_{\text{eq}}$), 3.95–3.85 (m, 3H, H-1' $_{\text{ax}}$, H-6', and H-6''), 3.19–3.16 (m, 1H, H-5'), 3.10–3.05 (m, 1H, H-4'), 2.66 (h, J = 6.9 Hz, 1H, CH- ^iPr), 2.30–2.26 (m, 1H, H-3' $_{\text{eq}}$), 1.75–1.69 (m, 1H, H-3' $_{\text{ax}}$), 1.26, 1.25 (2d, J = 6.9 Hz, 6H, $2 \times \text{CH}_3$ - ^iPr), 1.07 (s, 9H, $3 \times \text{CH}_3$ - ^iBu); ^{13}C NMR (125 MHz, CDCl_3) δ 178.4 (CO- ^iBu), 155.7 (C-6), 148.1 (C-2), 147.2 (C-4), 138.7 (C-8), 135.6, 135.6 (Ar C-TBDPS), 133.1, 132.9 ($2 \times ipso$ C-Bn), 129.9, 129.9, 127.8, 127.8 (Ar C-TBDPS), 120.7 (C-5), 84.4 (C-5'), 69.1 (C-1'), 64.1 (C-6'), 50.2 (C-2'), 43.7 (C-4'), 37.1 (C-3'), 36.5 (CH- ^iPr), 26.9 ($3 \times \text{CH}_3$ - ^iBu), 19.2 (1C- ^iBu), 19.0, 18.9 ($2 \times \text{CH}_3$ - ^iPr); HRMS (ESI-TOF) m/z : $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{31}\text{H}_{40}\text{N}_6\text{O}_4\text{Si}$, 589.2953; found, 589.2962.

1',5'-Anhydro-6'-O-tert-butylidiphenylsilyl-4'-amino(trityl)-3',2'-dideoxy-2'-(N²-iso-butrylguanin-9-yl)-D-alto-hexitol (51). A synthetic protocol similar to that used for the synthesis of 39 was employed for the synthesis of 51, starting from 50 (3.70 g, 6.28 mmol), trityl chloride (5.25 g, 18.85 mmol), and anhydrous triethylamine (8.73 mL, 62.8 mmol) in anhydrous CH_2Cl_2 (100 mL) to give 51 (5.11 g, 98%) as a white semisolid (R_f = 0.47, EtOAc; column chromatography gradient hexane/EtOAc, 4:1, v/v; 3:2, v/v;

1:4, v/v). ^1H NMR (500 MHz, CDCl_3) δ 11.83 (br s, 1H, NH-1), 7.99 (br s, 1H, NH-2), 7.61–7.57 (m, 4H, Ar H-Trt), 7.55 (s, 1H, H-8), 7.45–7.10 (m, 21H, Ar H-TBDPS and Trt), 4.37–4.33 (m, 1H, H-2'), 3.89 (dd, J = 12.3, 4.2 Hz, 1H, H-1' $_{\text{eq}}$), 3.80 (dd, J = 12.3, 3.5 Hz, 1H, H-1' $_{\text{ax}}$), 3.72 (dd, J = 11.3, 5.2 Hz, 1H, H-6'), 3.66 (dd, J = 12.3, 4.6 Hz, 1H, H-6''), 3.31–3.28 (m, 1H, H-5'), 2.66–2.62 (m, 1H, H-4'), 2.58 (h, J = 6.8 Hz, 1H, CH- ^iPr), 1.80–1.67 (m, 3H, H-3' $_{\text{eq}}$, H-3' $_{\text{ax}}$ and NH-4'), 1.29, 1.28 (2d, J = 6.9 Hz, 6H, $2 \times \text{CH}_3$ - ^iPr), 0.99 (s, 9H, $3 \times \text{CH}_3$ - ^iBu); ^{13}C NMR (150 MHz, CDCl_3) δ 177.9 (CO- ^iBu), 155.5 (C-6), 147.6 (C-2), 146.6 (C-4), 137.8 (C-8), 135.6, 135.5, 133.0, 132.9, 129.8, 129.7, 128.6, 127.7, 126.4 (Ar C-TBDPS and Trt), 121.0 (C-5), 81.0 (C-5'), 70.7 (1C-Trt), 67.4 (C-1'), 64.3 (C-6'), 49.2 (C-2'), 48.6 (C-4'), 36.6 (CH- ^iPr), 34.5 (C-3'), 26.8 ($3 \times \text{CH}_3$ - ^iBu), 19.1 (1C- ^iBu), 19.0, 18.9 ($2 \times \text{CH}_3$ - ^iPr); HRMS (ESI-TOF) m/z : $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{50}\text{H}_{54}\text{N}_6\text{O}_4\text{Si}$, 831.4048; found, 831.4048.

1',5'-Anhydro-4'-amino(trityl)-3',2'-dideoxy-2'-(N²-iso-butrylguanin-9-yl)-D-alto-hexitol (4). A synthetic protocol similar to that used for the synthesis of 3 was employed for the synthesis of 4, starting from 51 (5.1 g, 6.14 mmol) and triethylamine trihydrofluoride (4.5 mL, 27.61 mmol) in anhydrous THF (120 mL) at 46 °C for 60 h to obtain (R_f = 0.19, 10% MeOH in EtOAc; column chromatography gradient MeOH/EtOAc, 1:99, v/v; 1:49, v/v; 1:19, v/v) to give 50 (3.09 g, 85%) as a white semisolid. Mp 241 °C. ^1H NMR (500 MHz, $\text{DMSO}-d_6$) δ 12.08 (br s, 1H, NH-1), 11.59 (br s, 1H, NH-2), 7.77 (s, 1H, H-8), 7.33–7.07 (m, 15H, Ar H-Trt), 4.74 (t, J = 5.6 Hz, 1H, OH-6'), 4.36–4.33 (m, 1H, H-2'), 3.99 (d, J = 12.1 Hz, 1H, H-1' $_{\text{eq}}$), 3.82 (dd, J = 12.4, 2.9 Hz, 1H, H-1' $_{\text{ax}}$), 3.66–3.53 (m, 2H, H-6' and H-6''), 3.40–3.36 (m, 1H, H-5'), 3.02 (d, J = 6.5 Hz, 1H, NH-4'), 2.79 (h, J = 6.9 Hz, 1H, CH- ^iPr), 2.40–2.35 (m, 1H, H-4'), 1.64–1.58 (m, 1H, H-3' $_{\text{eq}}$), 1.46–1.42 (m, 1H, H-3' $_{\text{ax}}$), 1.16, 1.11 (2d, J = 6.8 Hz, 6H, $2 \times \text{CH}_3$ - ^iPr); ^{13}C NMR (125 MHz, $\text{DMSO}-d_6$) δ 180.2 (CO- ^iBu), 155.0 (C-6), 148.2 (C-2), 147.6 (C-4), 146.6 ($ipso$ C-Trt), 138.2 (C-8), 128.5, 127.5, 126.2 (Ar C-Trt), 119.7 (C-5), 81.2 (C-5'), 70.1 (1C-Trt), 67.5 (C-1'), 61.9 (C-6'), 49.3 (C-2'), 47.9 (C-4'), 34.7 (CH- ^iPr), 34.4 (C-3'), 19.0, 18.9 ($2 \times \text{CH}_3$ - ^iPr); HRMS (ESI-TOF) m/z : $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{34}\text{H}_{36}\text{N}_6\text{O}_4$, 593.2871; found, 593.2873.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.8b02444.

^1H , ^{13}C , APT, COSY, HSQC, HMBC, and HRMS spectra of intermediates and final compounds (PDF)

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Notes

The authors declare no competing financial interest.

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